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Abnormal striatal plasticity in a DYT11/SGCE myoclonus dystonia mouse model is reversed by adenosine A2A receptor inhibition☆

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ABSTRACT

Striatal dysfunction is implicated in many movement disorders. However, the precise nature of defects often remains uncharacterized, which hinders therapy development. Here we examined striatal function in a mouse model of the incurable movement disorder, myoclonus dystonia, caused by *SGCE* mutations. Using RNAseq we found surprisingly normal gene expression, including normal levels of neuronal subclass markers to strongly suggest that striatal microcircuitry is spared by the disease insult. We then functionally characterized *Sgce* mutant medium spiny projection neurons (MSNs) and cholinergic interneurons (ChIs). This revealed normal intrinsic electrophysiological properties and normal responses to basic excitatory and inhibitory neurotransmission. Nevertheless, high-frequency stimulation in *Sgce* mutants failed to induce normal long-term depression (LTD) at corticostriatal glutamatergic synapses. We also found that pharmacological manipulation of MSNs by inhibiting adenosine 2A receptors ($A_{2A}R$) restores LTD, again pointing to structurally intact striatal circuitry. The fact that *Sgce* loss specifically inhibits LTD implicates this neurophysiological defect in myoclonus dystonia, and emphasizes that neurophysiological changes can occur in the absence of broad striatal dysfunction. Further, the positive effect of $A_{2A}R$ antagonists indicates that this drug class be tested in DYT11/*SGCE* dystonia.

1. Introduction

Basal ganglia dysfunction is strongly implicated in dystonia, an incurable, disabling movement disorder characterized by abnormal postures and/or stereotyped movements (Albanese et al., 2013; Stacy, 2012). This includes forms of dystonia that lack a structural explanation for symptoms, and thus where a micro-pathology or a primary neurophysiological origin is suspected. However, dystonia remains poorly understood and there is insufficient information to design new therapies for this disease.

The striatum is the basal ganglia input nucleus that integrates information arriving from cortical and subcortical regions, and transmits to the next relay station, the globus pallidus (Alexander and Crutcher, 1990). In fact, deep brain stimulation (DBS) of the globus pallidus remains the most effective symptomatic treatment for many forms of dystonia (Vidailhet et al., 2013). The striatum is also where dopamine affects motor function, and indeed genetic mutations that reduce dopamine synthesis are sufficient to cause dystonia (DYT5/*GCH1* dystonia) (Clot et al., 2009; Ichinose et al., 1994). However, there is little evidence that dopamine deficiency is the cause of all dystonia since other

Abbreviations: ($A_{2A}R$), adenosine 2A receptors; (AHP), afterhyperpolarization; (ChI), cholinergic interneuron; (DBS), deep brain stimulation; (D1R), dopamine D1 receptor; (D2R), dopamine D2 receptor; (EPSP), excitatory postsynaptic potential; (HFS), high-frequency stimulation; (IR), input resistance; (LTD), long-term depression; (MSN), medium spiny projection neuron; (mGluR), metabotropic glutamate receptor; (mEPSC), miniature excitatory postsynaptic current; (mIPSC), miniature inhibitory postsynaptic current; (mAChR), muscarinic acetylcholine receptor; (PPR), paired-pulse ratio; (RMP), resting membrane potential; (sEPSC), spontaneous glutamatergic excitatory postsynaptic current; (sIPSC), spontaneous GABAergic inhibitory postsynaptic current; (TTX), tetrodotoxin.

☆ Conflict of interest: none to declare.

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dystonia-causing genes appear to act via different mechanisms. DYT25/*GNAL* mutations instead impair signal transduction in striatal projection neurons (Fuchs et al., 2012), while defects in the plasticity mechanisms that modulate striatal processing are implicated in DYT1/*TOR1A* dystonia (Arkadir et al., 2016; Kreitzer and Malenka, 2008; Martella et al., 2009).

The fact that different dystonia-causing mutations differentially affect the striatum raises the possibility that dystonia is a relatively non-specific consequence of disordered basal ganglia processing. On the other hand, insults that have distinct upstream effects may in fact converge to a single downstream target. If so, this point of convergence would be an important therapeutic target for a disease where new therapies are both lacking and needed. It is therefore interesting to consider that the striatal plasticity defects observed in DYT1/*TOR1A* and DYT5/*GCH1* dystonia are linked to dopamine levels and that the tone of striatal signal transduction is affected by DYT25/*GNAL* mutations (Kreitzer and Malenka, 2008; Lovinger, 2010). Thus, all these dystonia-causing insults acting on distinct targets could converge onto a common underlying mechanism of altered striatal plasticity with, as a consequence, abnormal striatal output.

We were therefore interested to examine a relatively poorly understood genetic dystonia, DYT11/*SGCE* myoclonus dystonia (OMIM 604149) (Kinugawa et al., 2009; Zimprich et al., 2001). *SGCE* is widely expressed and encodes the transmembrane ε-sarcoglycan protein that forms part of the dystrophin-glycoprotein complex. This complex is best characterized in muscle cells where it stabilizes the cell membrane against contraction-mediated damage (Sandona and Betto, 2009; Waite et al., 2009). Less is known about how ε-sarcoglycan operates in the CNS, although it is clear that the dystrophin complex helps organize post-synaptic machinery, particularly at inhibitory synapses (Pilgram et al., 2010; Waite et al., 2009), and has an evolutionarily conserved association with the strength of neurotransmission (Bogdanik et al., 2008; Fradkin et al., 2008; Pribiag et al., 2014; van der Plas et al., 2006). More clues to the origin of DYT11/*SGCE* myoclonus dystonia come from the other genes linked to this disease that include the *CACNA1B* calcium channel gene (Groen et al., 2015) and the *KCTD17* potassium channel tetramerization domain containing 17 gene (Mencacci et al., 2015); both again suggesting that myoclonus dystonia has a primary neurophysiological origin. However, despite this evidence, the role of *Sgce* in neuronal physiology is largely uncharacterized. Myoclonus dystonia is also associated with striatal dysfunction since symptoms respond well to basal ganglia DBS (Vidailhet et al., 2013) and striatal-specific *Sgce* deletion is described to alter motor behavior in mice (Yokoi et al., 2012). Thus, here we focus on how a disease-causing genetic insult in mouse *Sgce* impacts striatal function, focusing on the microcircuitry and neurophysiological properties of this nucleus.

2. Materials and methods

2.1. Creation and molecular characterization of a Sgce-Δexon5 allele

A targeting vector to remove ε-sarcoglycan expression was produced by cloning *Sgce* genomic sequence (spanning from GTTAAT GATGTGGCTAGAAGTTAGGG to GGCCCAGTAGATAGCAGGCGAG), inserting a loxP site immediately upstream of *Sgce* exon5 (following the sequence CCATTTTCTGGTGACA), and placing a loxP and FRT site flanked PGK-Neo cassette downstream of exon 5 (immediately following the sequence CAGCAGATGCCCTGCTGAG). The targeting vector was linearized with Not1 and electroporated into Hybrid C57Bl6/129SvEv embryonic stem cells (inGenious Targeting Laboratory). Cultures were selected with G418, surviving clones expanded, and homologous recombination between the targeting vector and *Sgce* detected by PCR and Southern blotting. PCR across exon5 and surrounding regions was used to confirm the presence of the distal loxP site. ES cells were then microinjected into blastocysts and implanted into foster mice. Resulting chimeric offspring (F0) were crossed with Flp-deleter mice (inGenious Targeting Laboratory) and tail biopsies from F1 offspring examined for a) the deletion of the PGK-Neo cassette and b) presence of loxP sites; both by amplification of an ~900 bp band using forward primer: ACCAGTACAACCATAGCATG and reverse primer: ATTGTCTACCTCAA GTCCCC (Fig. 1A & B). Finally *Sgce* exon 5 sequence was deleted by interbreeding 'exon5 flox' F1 mice with EIIa-Cre mice (Lakso et al., 1996), and the successful removal of exon5 from F2 progeny confirmed by the same PCR reaction detecting a 400 bp deletion compared to the wild-type allele (Fig. 1B). Experimental animals were generated by mating heterozygous male $Sgce^{+/\Delta ex5}$ (also tested negative by PCR for Cre and Flp transgenes) with wild-type C57Bl6 females. This crossing scheme produces equal numbers of littermate control ($Sgce^{+/+}$) and disease model mice ($Sgce^{+/-}$) that have a paternally inherited mutant *Sgce* allele.

Western blotting was performed with lung and sciatic nerve samples dissected from adult animals after cervical dislocation. Blots loaded with equal amounts of protein were probed with a rabbit anti ε-sarcoglycan antibody raised against a recombinant cytosolic domain fragment from type-4 mouse ε-sarcoglycan (NP_035490) as previously described (Jungwirth et al., 2010; Nishiyama et al., 2004). All animal experiments were approved by the ethical committees of KU Leuven and UZ Leuven (LA1210596) and/ or the Animal Care and Use Committee of University of Rome "Tor Vergata". Animal experiments were also carried out in accordance with EC, Internal Institutional Review Committee, EU directive and Italian rules (63/2100 EU; Italian law: DLS/26 04/03/2014). All members of the staff involved in the experiments have undergone specific training for handling and working with rodents.

2.2. Transcriptomics

Animals were euthanized by cervical dislocation, brains rapidly removed, chilled, and coronal 1 mm forebrain slices collected using a prechilled brain matrix. The striatum was then sub-dissected from slices and frozen in liquid nitrogen. Once all striata were collected, RNA was extracted and purified using Trizol reagent. RNA concentration and purity were determined spectrophotometrically using the Nanodrop ND-1000 (Nanodrop Technologies) and RNA integrity assessed using a Bioanalyser 2100 (Agilent). For samples with a 260/230 ratio below 1.8, 1 μg total RNA was precipitated with ethanol. Library preparation, sequencing and statistical analysis were performed by VIB Nucleomics Core (www.nucleomics.be). Each library was prepared from 500 ng total RNA using the Illumina TruSeq® Stranded mRNA Sample Prep Kit (protocol version October 2013, Rev. E), poly-A containing mRNA molecules were purified from the total RNA input using poly-T oligo-attached magnetic beads. In a reverse transcription reaction using random primers, RNA was converted into first strand cDNA and subsequently converted into double-stranded cDNA in a second strand cDNA synthesis reaction using DNA Polymerase I and RNAse H. The cDNA fragments were extended with a single 'A' base to the 3′ ends of the blunt-ended cDNA fragments after which multiple indexing adapters were ligated introducing different barcodes for each sample. Finally, enrichment PCR was carried out to enrich those DNA fragments that have adapter molecules on both ends and to amplify the amount of DNA in the library, with the following minor modifications: only half of the library is subjected to a 13 cycle PCR in the final library amplification step. These sequence-libraries of each sample were pooled at equal molarity and sequenced with a 1 x 50 bp kit on 2 lanes of a HiSeq2000 flow-cell.

2.2.1. Preprocessing

Low quality ends and adapter sequences were trimmed off from the Illumina reads with FastX 0.0.13 and Cutadapt 1.2.1. Using FastX 0.0.13 and ShortRead 1.16.3, we subsequently filtered small reads (length < 35 bp), polyA-reads (>90% of the bases equal A), ambiguous reads (containing N), and low-quality reads (>50% of the bases <Q25). With