Neurobiology of Disease 97 (2017) 46–59

Contents lists available at ScienceDirect

Neurobiology of Disease

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

The sigma-1 receptor mediates the beneficial effects of pridopidine in a mouse model of Huntington disease

Daniel Ryskamp [a], Jun Wu [a], Michal Geva [b], Rebecca Kusko [c], Iris Grossman [b], Michael Hayden [b,*], Ilya Bezprozvanny [a,**]

[a] Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
[b] Teva Pharmaceutical Industries, 5 Basel St., Petach Tikva 49131, Israel
[c] Immuneering Corporation, Cambridge, MA 02142, USA

ARTICLE INFO

Article history:
Received 16 May 2016
Revised 13 September 2016
Accepted 30 October 2016
Available online 3 November 2016

Keywords:
Huntington disease
Pridopidine
3-PPP
Sigma-1 receptor
Medium spiny neurons
YAC128 mice
Corticostriatal co-culture
Synaptic instability
Store-operated calcium entry

ABSTRACT

The tri-nucleotide repeat expansion underlying Huntington disease (HD) results in corticostriatal synaptic dysfunction and subsequent neurodegeneration of striatal medium spiny neurons (MSNs). HD is a devastating autosomal dominant disease with no disease-modifying treatments. Pridopidine, a postulated "dopamine stabilizer", has been shown to improve motor symptoms in clinical trials of HD. However, the target(s) and mechanism of action of pridopidine remain to be fully elucidated. As binding studies identified sigma-1 receptor (S1R) as a high-affinity receptor for pridopidine, we evaluated the relevance of S1R as a therapeutic target of pridopidine in HD. S1R is an endoplasmic reticulum - (ER) resident transmembrane protein and is regulated by ER calcium homeostasis, which is perturbed in HD. Consistent with ER calcium dysregulation, we observed striatal upregulation of S1R in aged YAC128 transgenic HD mice and HD patients. We previously demonstrated that dendritic MSN spines are lost in aged corticostriatal co-cultures from YAC128 mice. We report here that pridopidine and the chemically similar S1R agonist 3-PPP prevent MSN spine loss in aging YAC128 co-cultures. Spine protection was blocked by neuronal deletion of S1R. Pridopidine treatment suppressed supranormal ER Ca^{2+} release, restored ER calcium levels and reduced excessive store-operated calcium (SOC) entry in spines, which may account for its synaptoprotective effects. Normalization of ER Ca^{2+} levels by pridopidine was prevented by S1R deletion. To evaluate long-term effects of pridopidine, we analyzed expression profiles of calcium signaling genes. Pridopidine elevated striatal expression of calbindin and homer1a, whereas their striatal expression was reduced in aged Q175KI and YAC128 HD mouse models compared to WT. Pridopidine and 3-PPP are proposed to prevent calcium dysregulation and synaptic loss in a YAC128 corticostriatal co-culture model of HD. The actions of pridopidine were mediated by S1R and led to normalization of ER Ca^{2+} release, ER Ca^{2+} levels and spine SOC entry in YAC128 MSNs. This is a new potential mechanism of action for pridopidine, highlighting S1R as a potential target for HD therapy. Upregulation of striatal proteins that regulate calcium, including calbindin and homer1a, upon chronic therapy with pridopidine, may further contribute to long-term beneficial effects of pridopidine in HD.

1. Introduction

Huntington disease (HD) is a progressive neurodegenerative disease resulting from a dominantly-inherited trinucleotide (CAG) repeat expansion in the huntingtin gene, encoding polyglutamine-expanded mutant Huntingtin (mHtt) protein (MacDonald et al., 1993). HD symptoms, which include motor, cognitive and psychiatric disturbances, typically present around 40 years of age and progressively worsen until death approximately 20 years after diagnosis (Foroud et al., 1999). HD management is limited to supportive care and symptomatic treatment (Bates et al., 2015). Pridopidine (ACR16) is emerging in clinical trials as a potential therapeutic to mitigate motor symptoms (*e.g.*, total motor score improvement was observed when tested as a secondary

Abbreviations: HD, Huntington disease; Htt, Huntingtin; MSN, medium spiny neuron; S1R, sigma-1 receptor; ER, endoplasmic reticulum; YAC128, yeast artificial chromosome 128Q expansion mice; 3-PPP, R(+)-3-(3-Hydroxyphenyl)-*N*-propylpiperidine hydrochloride; SOC, store-operated calcium current; MAM, mitochondrial-associated membrane; gLacZ, guideRNA targeting the bacterial β-galactosidase gene; gS1R, guideRNA targeting the mouse S1R gene; MEF, mouse embryonic fibroblasts; DHPG, (*S*)-3,5-dihydroxyphenylglycine; IO, ionomycin.

* Correspondence to: M. Hayden, 5 Basel St., Petach Tikva 49131, Israel.
** Correspondence to: I. Bezprozvanny, 5323 Harry Hines Blvd., ND12.200, Dallas, TX 75390, USA.
E-mail addresses: Daniel.Ryskamp@UTSouthwestern.edu (D. Ryskamp), Jun.Wu@UTSouthwestern.edu (J. Wu), Michal.Geva@teva.co.il (M. Geva), BKusko@immuneering.com (R. Kusko), Iris.Grossman@teva.co.il (I. Grossman), Michael.Hayden@teva.co.il (M. Hayden), Ilya.Bezprozvanny@UTSouthwestern.edu (I. Bezprozvanny).

Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2016.10.006
0969-9961/

endpoint in two independent clinical trials) in HD patients (de Yebenes et al., 2011; Esmaeilzadeh et al., 2011; Huntington Study Group, 2013; Lundin et al., 2010). Pridopidine was initially identified as a stabilizer of the dopamine system, normalizing hyper- and hypodopaminergic behaviors, with the proposed mode of action of a D_2 receptor (D2R) antagonist, a partial weak agonist, or both a positive allosteric modulator and an orthosteric antagonist (Dyhring et al., 2010; Nilsson et al., 2004; Rung et al., 2008). However, the affinity of pridopidine for D2R is low (IC50 and Ki ~10 μM) (Dyhring et al., 2010) compared to its affinity for the sigma-1 receptor (S1R; Ki ~80 nM) (Sahlholm et al., 2013). Indeed, pridopidine exhibits efficient S1R binding, but not D2R binding, at behaviorally relevant doses *in vivo* (Sahlholm et al., 2015), indicating that the therapeutic mechanism of action for pridopidine may primarily involve the S1R.

S1R is a brain-enriched, transmembrane protein of 223 amino acids in the endoplasmic reticulum (ER) (Kourrich et al., 2012). S1R is evolutionarily conserved and lacks sequence homology with other mammalian proteins. Computational modeling and NMR studies indicate that S1R contains 2 transmembrane domains in ER membrane (Brune et al., 2014; Ortega-Roldan et al., 2015), although a recent crystal structure indicated a single transmembrane domain topology (Schmidt et al., 2016). S1R is often referred to as a "chaperone" (Su et al., 2010), but its primary function appears to involve modulation of ion channels (Kourrich et al., 2012). S1R is normally restricted to mitochondrial-associated membrane (MAM) domains where it regulates calcium (Ca^{2+}) signaling between the ER and mitochondria, as well as lipid transport (Hayashi and Su, 2003; Hayashi and Su, 2007). However, high concentrations of S1R agonists, or alternatively ER stress, lead to dislocation of S1R beyond the MAM domain (Su et al., 2010) so as to regulate ion channels on the plasma membrane (Kourrich et al., 2012). Other roles have been reported for S1R in brain function, including neuromodulation (Maurice et al., 2006) and neuroplasticity (Kourrich et al., 2012; Takebayashi et al., 2004; Tang et al., 2009; Tsai et al., 2009).

S1R was first identified as a target for treating neuropsychiatric disorders, including drug addiction, depression and schizophrenia (Maurice and Su, 2009). Additional indications are now emerging from genetic data pertaining to neurodegenerative diseases, such as Alzheimer's disease (Fehér et al., 2012; Mishina et al., 2008; Uchida et al., 2005), amyotrophic lateral sclerosis (Al-Saif et al., 2011), hereditary motor neuropathy (Li et al., 2015) and frontotemporal lobar degeneration (Luty et al., 2010). Several studies have identified neuroprotective properties of S1R modulators (Fisher et al., 2016; Marrazzo et al., 2005; Ruscher et al., 2011; Schetz et al., 2007; Smith et al., 2008). In previous studies, the S1R agonist PRE-084 displayed neuroprotective properties in PC6.3 cells expressing N-terminal mHtt (Hyrskyluoto et al., 2013). Similarly, pridopidine improved motor performance and prolonged survival of R6/2 HD mice and exerted neuroprotective effects in a mouse striatal knock-in cellular model of HD ($STHdh^{111/111}$) (Squitieri et al., 2015). These data suggest that pridopidine might act as a disease-modifying therapeutic in HD by stimulating S1R activity.

Early neuropathological features of HD include perturbed corticostriatal synaptic function and connectivity (Miller and Bezprozvanny, 2010; Milnerwood and Raymond, 2007; Milnerwood and Raymond, 2010; Murmu et al., 2013; Orth et al., 2010; Schippling et al., 2009), eventually leading to overt neurodegeneration of medium spiny neurons (MSNs) in the striatum (Myers et al., 1988; Vonsattel and DiFiglia, 1998). Perturbed stability of synaptic spines has been suggested to underlie the development of HD symptoms (Bezprozvanny and Hiesinger, 2013; Murmu et al., 2013; Ryskamp et al., 2016). In recent studies, we demonstrated that post-synaptic dendritic spines of MSNs are lost in aged corticostriatal co-cultures established from YAC128 mice (Wu et al., 2016). In the present study, we used this *in vitro* HD MSN spine loss model to investigate the potential mechanism of action of pridopidine in HD and to assess S1R as a therapeutic target of pridopidine.

2. Materials and methods

2.1. Mice

Experiments involving mice were approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center at Dallas and followed the National Institutes of Health Guidelines for the Care and Use of Experimental Animals. Wild type (WT; FVB/NJ) and YAC128 transgenic (FVB-Tg(YAC128)53Hay/J; Jackson Labs: stock # 004938) mice (Slow et al., 2003) were maintained at UT Southwestern Medical Center in barrier facility (12 h light/dark cycle) and genotyped as in (Wu et al., 2011).

2.2. Statistical analysis of the Q175 HD mouse model gene expression (CHDI allelic series six month dataset)

CAG knockin mouse RNAseq data were downloaded from the Cure Huntington's Disease Initiative (CHDI) website http://chdifoundation.org/datasets/ and were normalized using the voom transform from R package limma v3.18.13 in R v3.1.3. Using lmFit, genes were tested for differential expression in striata comparing between 6 months old Q20 (normal phenotype) and 6 months old Q175 heterozygotes. Q175 mice express an allele encoding the human HTT exon 1 sequence with a ~190 CAG repeat tract that replaces mouse Htt exon 1 and results in an HD phenotype.

2.3. Chronic treatment animal studies of gene expression profiles

Gene expression analysis of pridopidine-treated rats was recently described (Geva et al., 2016). Briefly, Sprague Dawley rats ($n = 6$) were treated daily by oral gavage with pridopidine (60 mg/kg) over 10 days. Six control Sprague Dawley rats were vehicle-treated. On the 10th day, 90 min following the last drug administration, brains were removed and RNA was isolated from the striatum of each rat and was analyzed using Affymetrix Rat 230_2 arrays. The gene expression data from 12 striatum samples was RMA normalized with affy package v1.42.3 in R v3.1.2. Probesets were annotated according to the Affymetrix Rat230_2 Release 22 annotation file. Processing of the Affymetrix data is detailed in the previous publication (Geva et al., 2016). The limma package v3.18.13 in R v3.1.3 was used to test if relevant calcium-related genes were differentially expressed between the two groups of biological replicates and multiple hypothesis testing was corrected for using the Bonferroni correction. Limma employs an empirical Bayes method to moderate standard error (Ritchie et al., 2015). When a gene had multiple probesets, the probeset with the highest absolute value of fold change was reported.

2.4. Western blot analysis

Striata from WT and YAC128 mice at 2, 6, and 12 months of age were isolated by dissection in PBS following euthanasia by euthasol injection and cervical dislocation. Cortices were similarly isolated for WT and YAC128 mice at 12 months of age. Human samples from caudate/nucleus accumbens/putamen and globus pallidus were obtained from the Harvard Brain Tissue Resource Center (http://www.brainbank.mclean.org/) and were processed as in (Sun et al., 2014). Isolated tissue was weighed and for each 100 mg of tissue, 200 μl of cold lysis buffer (1% CHAPS, 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.2, 5 mM EDTA, 5 mM EGTA, 1 mM PMSF, 50 mM NaF, 1 mM Na_3VO_4 and protease inhibitors) was used for homogenizing tissue and solubilizing protein (4 °C for 1 h). Samples were centrifuged at 10,000*g* for 10 min at 4 °C and the supernatant was transferred to a new tube. For human samples, the protein concentration was measured with a NanoDrop. Based on the volume of supernatant, an appropriate amount of 6× SDS buffer was added to each sample. Human samples were diluted with 1× SDS buffer based on the measured concentration