Developmental Biology 394 (2014) 24–38

Contents lists available at ScienceDirect

Developmental Biology

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

Huntingtin regulates Ca^{2+} chemotaxis and K^+-facilitated cAMP chemotaxis, in conjunction with the monovalent cation/H^+ exchanger Nhe1, in a model developmental system: Insights into its possible role in Huntington's disease

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ARTICLE INFO

Article history:
Received 17 April 2014
Received in revised form
7 July 2014
Accepted 7 August 2014
Available online 19 August 2014

Keywords:
Huntingtin
Actin-myosin cytoskeleton
Myosin heavy chain kinase
Inositol 1,4,5-trisphosphate receptor
Cation/H^+ exchanger
Calcium chemotaxis
Nhe1
Cell motility

ABSTRACT

Huntington's disease is a neurodegenerative disorder, attributable to an expanded trinucleotide repeat in the coding region of the human *HTT* gene, which encodes the protein huntingtin. These mutations lead to huntingtin fragment inclusions in the striatum of the brain. However, the exact function of normal huntingtin and the defect causing the disease remain obscure. Because there are indications that huntingtin plays a role in Ca^{2+} homeostasis, we studied the deletion mutant of the *HTT* ortholog in the model developmental system *Dictyostelium discoideum*, in which Ca^{2+} plays a role in receptor-regulated behavior related to the aggregation process that leads to multicellular morphogenesis. The *D. discoideum* *htt*$^-$-mutant failed to undergo both K^+-facilitated chemotaxis in spatial gradients of the major chemoattractant cAMP, and chemotaxis up a spatial gradient of Ca^{2+}, but behaved normally in Ca^{2+}-facilitated cAMP chemotaxis and Ca^{2+}-dependent flow-directed motility. This was the same phenotypic profile of the null mutant of Nhel, a monovalent cation/H^+exchanger. The *htt*$^-$-mutant also failed to orient correctly during natural aggregation, as was the case for the Nhel mutant. Moreover, in a K^+-based buffer the normal localization of actin was similarly defective in both *htt*$^-$ and *nhe1*$^-$ cells in a K^+-based buffer, and the normal localization of Nhe1 was disrupted in the *htt*$^-$ mutant. These observations demonstrate that *Htt* and Nhel play roles in the same specific cation-facilitated behaviors and that Nhel localization is directly or indirectly regulated by *Htt*. Similar cation-dependent behaviors and a similar relationship between Htt and Nhe1 have not been reported for mammalian neurons and deserves investigation, especially as it may relate to Huntington's disease.

Introduction

Huntington's disease (HD), formerly known as Huntington's chorea (Huntington, 1872), is an autosomal dominant neurodegenerative disorder, resulting from a CAG trinucleotide repeat expansion mutation in the huntingtin gene (*HTT*). This results in a longer dysfunctional version of a normally polymorphic polyglutamine segment (polyQ > 37 residues) at the amino-terminus of the huntingtin protein (The Huntington's Disease Collaborative Research Group, 1993; Gusella and MacDonald, 1995; DiFiglia et al., 1995). Mutated huntingtin eventually forms aggregates within medium spiny neurons in the striatum of the brain (Hsiao and Chern, 2010) and results in progressive neuronal loss, motor disturbances, dementia, involuntary muscle spasms and death (The Huntington's Disease Collaborative Research Group, 1993; Gusella and MacDonald, 1995; DiFiglia et al., 1995).

Huntingtin appears to be involved in a number of functions (Cattaneo et al. 2005; Imarisio et al., 2008). However, the embryonic lethality associated with loss of the huntingtin gene in the mouse (Nasir et al., 1995) complicates attempts to understand the basic function of huntingtin and the exact malfunctions contributing to HD. Because of the evolutionary conservation of the huntingtin gene, lower eukaryotic systems provide the potential for understanding huntingtin function. Consequently, the model developmental organism *D. discoideum* (Williams et al., 2006;

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http://dx.doi.org/10.1016/j.ydbio.2014.08.009
0012-1606/

Myre, 2012; Müller-Taubenberger et al., 2013; Muñoz-Braceras et al., 2013; Annesley et al., 2014), which undergoes cellular locomotion and chemotaxis in a manner similar to human cells (Cai and Devreotes, 2011; Wang et al., 2011a; Jin, 2013), has been used to explore the role of huntingtin at the cellular level.

Wang et al. (2011b) and Myre et al. (2011) independently disrupted *htt,* the ortholog of human *HTT*, in *D. discoideum*. Normally, populations of *D. discoideum* amoebae, when starved for nutrients on a substrate, aggregate by undergoing chemotaxis in response to relayed outwardly moving, nondissipating waves of the chemoattractant cAMP, released in a pulsatile fashion from aggregation centers (Konijin et al., 1967; Gerisch et al., 1975; Tomchik and Devreotes, 1981). Chemotactic orientation in the increasing spatial gradient of cAMP in the front of each relayed wave in a natural aggregation territory is facilitated by chemotaxis up steep transient Ca^{2+} gradients generated between cells in response to the onset of the front of each cAMP wave (Scherer et al., 2010). Both Wang et al. (2011b) and Myre et al. (2011) showed that *htt*$^-$ cells were defective in chemotaxis in a spatial gradient of cAMP generated *in vitro*.

Interestingly, the behavioral defects of *htt*$^-$ cells were partially rescued by raising the extracellular concentration of Ca^{2+} (Myre et al., 2011). We previously had found that optimal chemotactic orientation of *D discoideum* cells in a cAMP gradient generated *in vitro*, required a threshold concentration of either the monovalent cation K^+ or the divalent cation Ca^{2+} (Lusche et al., 2009; Lusche et al., 2011). Furthermore, we found that Nhe1, a monovalent cation/H^+ exchanger at the plasma membrane (Patel and Barber, 2005), was essential for K^+-facilitated, but not Ca^{2+}-facilitated chemotaxis in a cAMP gradient (Lusche et al., 2011). We also found that wild type *D. discoideum* cells undergo positive chemotaxis in spatial gradients of Ca^{2+} generated *in vitro* (Scherer et al., 2010). These studies (Lusche et al., 2009, 2011, 2012; Soll et al., 2011; Wessels et al., 2012) and others showing that cAMP induces the release of Ca^{2+} (Bumann et al., 1986; Bohme et al., 1987), led us to the hypothesis that the onset of the front of each cAMP wave during natural aggregation induces transient Ca^{2+} gradients between cells that augment the accuracy of orientation towards the aggregation center (Scherer et al., 2010; Lusche et al., 2012; Wessels et al., 2012).

In our efforts to dissect the Ca^{2+} chemotaxis pathway, we discovered that the inositol 1,4,5-trisphosphate receptor-like protein IplA, a putative Ca^{2+} channel with homology to the mammalian inositol (1,4,5) trisphosphate receptors, $InsP_3R$ (Traynor et al., 2000), was essential for chemotaxis in a spatial gradient of Ca^{2+} and, as previously shown, was essential for Ca^{2+}-dependent flow-directed motility (Lombardi et al., 2008), but was not essential for cAMP chemotaxis facilitated by Ca^{2+} or K^+ (Lusche et al., 2012). We also found that cells lacking myosin heavy chain kinase A (*mhckA*$^-$) or myosin heavy chain kinase C (*mhckC*$^-$), were behavioral phenocopies of the mutant *iplA*$^-$ – *i.e.*, they exhibited normal chemotaxis in spatial gradients of cAMP, but were unable to undergo chemotaxis in spatial gradients of Ca^{2+} (Wessels et al., 2012). This led us to hypothesize that Ca^{2+} chemotaxis and cAMP chemotaxis were regulated by different signal transduction pathways beginning with different receptors and targeting different myosin heavy chain kinases. In support of our hypothesis that the pathway for Ca^{2+} chemotaxis functions in normal aggregation, we found that the three mutants which failed to undergo Ca^{2+} chemotaxis *in vitro* (*iplA*$^-$, *mhckA*$^-$ and *mhckC*$^-$), all exhibit a diminished capacity to accurately orient in the front of successive cAMP waves during natural aggregation (Lusche et al., 2012; Wessels et al., 2012). All three of these mutants also lost Ca^{2+}-dependent flow-directed motility (Lusche et al., 2012; Wessels et al., 2012).

Ca^{2+} dysregulation has been documented in several neurodegenerative diseases (Mattson, 2007), including HD (Zundorf and Reiser, 2011; Giacomello et al., 2011), and the Ca^{2+}-release channel inositol-(1,4,5) trisphosphate receptor 1 ($InsP_3R1$) has been implicated in the disorder (Tang et al., 2009; Kaltenbach et al., 2007; Matsumoto et al., 1996; Street et al., 1997; Brezprozvanny, 2011). We therefore considered the hypothesis that huntingtin played a role in Ca^{2+} chemotaxis, Ca^{2+}-dependent flow-directed motility, and Ca^{2+}-facilitated cAMP chemotaxis. To explore this hypothesis, we tested the capacity of *htt*$^-$ cells to undergo four behaviors that are dependent on cations and involve specific receptors, ions channels and/or monovalent cation exchangers. The four include (1) Ca^{2+}-facilitated cAMP chemotaxis, which involves the cAMP receptor (Klein et al., 1988) and an unidentified Ca^{2+} channel (Schaloske et al., 2005); (2) K^+-facilitated cAMP chemotaxis, which involves the cAMP receptor and the monovalent cation/H^+ exchanger Nhe1 (Patel and Barber, 2005; Lusche et al., 2011); (3) Ca^{2+}-dependent flow-directed motility, which, as shown here, involves Nhe1 and, as previously shown, IplA (Fache et al., 2005; Lusche et al., 2012); and (4) Ca^{2+} chemotaxis (Scherer et al., 2010), which also involves IplA (Lusche et al., 2012) and, as shown here, Nhe1. We demonstrate here that huntingtin is essential for only two of these four cation-dependent behaviors, namely, K^+-facilitated cAMP chemotaxis and Ca^{2+} chemotaxis, both of which also require Nhe1. Ca^{2+}-dependent flow-directed motility requires IplA, but as we show here, is independent of Nhe1. Our results suggest that in *D. discoideum*, the function of huntingtin is selectively intertwined directly or indirectly with that of Nhe1 in two behavioral responses involving different receptors and, surprisingly, different downstream myosin heavy chain kinases (Wessels et al., 2012). *Htt* plays no role in two additional responses involving the same alternative receptors. In support of the observed functional association of Nhe1 and Htt, actin localization was similarly defective in the *nhe1*$^-$ and *htt*$^-$ mutants (Patel and Barber, 2005; observations presented here), and localization of Nhe1 was abnormal in the *htt*$^-$ mutant. To our knowledge, neither a relationship between huntingtin and select receptor-mediated chemotaxis systems, nor a relationship between huntingtin and a monovalent cation/H^+ exchanger, has been reported for mammalian cells, and therefore deserves scrutiny, especially as these observations relate to HD.

Results

Huntingtin is not essential for Ca^{2+}-facilitated cAMP chemotaxis

To assess whether huntingtin is involved in chemotaxis of *D. discoideum* amoebae in a shallow gradient of cAMP, in which Ca^{2+} is the facilitating cation (Lusche et al., 2009, 2011), control Ax3 and *htt*$^-$ cells were developed on filter pads saturated with MES buffer containing 10 mM Ca^{2+} for 7 and 9 h, respectively, to achieve chemotactic responsiveness (Lusche et al., 2009, 2011). Cells were then dispersed on the bridge of a gradient chamber (Zigmond, 1977; Varnum and Soll, 1984) (Fig. 1A) in which a solution of 10^{-6} M cAMP in tricine buffer containing 10 mM Ca^{2+} (Ca^{2+}–tricine buffer) was placed in the "source" well and a solution of Ca^{2+}–tricine buffer lacking cAMP was placed in the "sink" well (Fig. 1A) (Lusche et al., 2011). Ca^{2+} at a concentration of 10 mM in buffer containing a non-facilitating concentration of monovalent cations has been shown to facilitate efficient chemotaxis of wild type cells in a spatial gradient of cAMP generated *in vitro* (Lusche et al., 2009, 2011). Two independently isolated *htt*$^-$ strains, *htt*$^-$a and *htt*$^-$b, were compared to parental Ax3 cells (controls) under these conditions. Cells of both *htt*$^-$ strains moved in a persistent fashion (Fig. 1D) up the gradient, in a manner similar to that of parental Ax3 cells (Fig. 1C), at comparable mean instantaneous velocities (Fig. 1E), and only slightly reduced