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Inositol 1,4,5-triphosphate receptor, calcium signaling and polyglutamine expansion disorders

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Abstract

Huntington's disease (HD), spinocerebellar ataxia type 2 (SCA2) and type 3 (SCA3) are autosomal-dominant neurodegenerative disorders. HD is caused by polyglutamine (polyQ) expansion (exp) in the amino-terminal region of a protein huntingtin (Htt) and primarily affects medium spiny striatal neurons (MSN). SCA2 is caused by a polyQ expansion in the amino-terminal region of a cytosolic protein ataxin-2 (Atxn2) and primarily affects cerebellar Purkinje neurons. SCA3 is caused by a polyQ expansion in the carboxy-terminal portion of a cytosolic protein ataxin-3 (Atxn3) and primarily affects dentate and pontine nuclei and substantia nigra. The reasons for neuronal dysfunction and death in HD, SCA2 and SCA3 remain poorly understood and no cure is available for the patients. My laboratory discovered that mutant Huntingtin, ataxin-2 and ataxin-3 proteins specifically binds to the carboxy-terminal region of the type 1 inositol 1,4,5-trisphosphate receptor (IP₃R1), an intracellular Ca²⁺ release channel. Moreover, we found that association with IP₃R1 causes sensitization of IP₃R1 to activation by IP₃ in planar lipid bilayers and in neuronal cells. These results suggested that deranged neuronal Ca²⁺ signaling might play an important role in pathogenesis of HD, SCA2 and SCA3. In support of this idea, we demonstrated a connection between abnormal Ca²⁺ signaling and neuronal cell death in experiments with HD, SCA2 and SCA3 transgenic mouse models. Based on these results I propose that IP₃R and other Ca²⁺ signaling proteins should be considered as potential therapeutic targets for treatment of HD, SCA2 and SCA3.

Keywords

calcium signaling; huntingtin; neurodegeneration; polyglutamine expansion; inositol 1,4,5-trisphosphate; ataxin-2; ataxin-3; spinocerebellar ataxias; transgenic mice; NMDA receptor; apoptosis; mitochondria; memantine

I. Huntington disease (HD), spinocerebellar ataxia type 2 (SCA2) and spinocerebellar ataxia type 3 (SCA3)

Huntington disease (HD) is an autosomal-dominant neurodegenerative disorder with the age of onset between 35 and 50 years and inexorable progression to death 15-20 years after

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onset. The symptoms include motor abnormalities including chorea and psychiatric disturbance with gradual dementia (Vonsattel and DiFiglia, 1998). Neuropathological analysis reveals selective and progressive neuronal loss in the striatum (caudate nucleus, putamen and globus pallidus) (Vonsattel et al., 1985; Vonsattel and DiFiglia, 1998). GABAergic medium spiny striatal neurons (MSN) are the most sensitive to neuronal degeneration in HD (Vonsattel et al., 1985; Vonsattel and DiFiglia, 1998). Positional cloning efforts demonstrated that at the molecular level the cause of HD is polyglutamine (polyQ) expansion in the amino-terminal of a 350 kDa evolutionary conserved cytosolic protein called huntingtin (Htt) (1993). Clinical signs of HD develop if the length of polyQ track in Htt exceeds a pathological threshold of 35Q. The CAG repeat length is inversely correlated with age of onset (Langbehn et al., 2004). Htt is widely expressed in the brain and in non-neuronal tissues and not particularly enriched in the striatum (Li et al., 1993; Strong et al., 1993; Sharp et al., 1995). Htt plays an essential function in development, as deletion of the Htt gene in mice is embryonic lethal (Duyao et al., 1995; Nasir et al., 1995). Analysis of Htt primary sequence suggests that Htt is likely to function as a signaling scaffold (MacDonald, 2003), but the precise function of Htt in cells is not known. In order to elucidate the pathogenesis of HD, a number of transgenic HD mouse models have been generated (Menalled and Chesselet, 2002; Rubinsztein, 2002).

Spinocerebellar ataxias (SCAs) constitute a heterogeneous group of autosomal-dominant genetic and neurodegenerative disorders. SCAs are generally characterized by cerebellar atrophy and a progressive incoordination of movement known as ataxia (Filla et al., 1999; Schols et al., 2004; Lastres-Becker et al., 2008). A total of 29 SCAs have been identified and named in the chronological order of their discovery from SCA1 to SCA30. SCA15 and SCA16 are allelic (Hara et al., 2008; Iwaki et al., 2008). Although all SCA patients present with the phenotypic overlap of cerebellar atrophy and ataxia, other brain regions are differentially affected in each SCA. Seventeen genes have been associated with these SCAs and it is not understood how mutations in those SCA-associated genes lead to the SCA pathogenesis (Paulson, 2009). The pathogenesis of SCAs is not fully understood, however, several different pathogenic mechanisms have been studied in SCAs such as dysregulation of transcription and gene expression, alterations in calcium homeostasis and synaptic neurotransmission, mitochondrial stress and apoptosis (reviewed in (Carlson et al., 2009; Matilla-Duenas et al., 2009; Bezprozvanny and Klockgether, 2010)). Currently, therapy for SCA patients is mainly supportive and directed at treating individual symptoms in each patient using methods such as deep brain stimulation and levodopa supplements (Pirker et al., 2003; Bezprozvanny and Klockgether, 2010). No disease modifying therapy exists for any of the SCAs. In order to develop a successful treatment of SCAs, it will be important that a valid therapeutic target and the pathogenic pathways are identified.

In this chapter I discuss recently emerging results that support the concept that excessive activity of inositol (1,4,5)-triphosphate receptors HD and abnormal Ca^{2+} signaling is playing a major role in pathogenesis of HD, SCA2 and SCA3.

II. Mutant Huntingtin specifically sensitizes IP₃R1 to IP₃

The inositol (1,4,5)-triphosphate receptor (IP₃R) is an intracellular calcium (Ca²⁺) release channel that plays an important role in neuronal Ca²⁺ signaling (Berridge, 1998). Three isoforms of IP₃R have been identified (Furuichi et al., 1994). The type 1 receptor (IP₃R1) is the predominant neuronal isoform. Mice lacking IP₃R1 display severe ataxic behavior (Matsumoto et al., 1996), and mice with a spontaneous mutation in the *IP₃R1* gene experience convulsions and ataxia (Street et al., 1997), suggesting a major role of IP₃R1 in neuronal function. Our laboratory has been heavily invested in elucidating the interaction between mHtt and IP₃, as well as other Ca²⁺-related mechanisms relevant to HD (for reviews see (Bezprozvanny and Hayden, 2004; Bezprozvanny, 2009)). We first discovered that mHtt binds directly and specifically to the C-terminal region of the type 1 IP₃ receptor (IP₃R1) (Tang et al., 2003). Recently, unbiased high-throughput screening assays confirmed mHtt binding to IP₃R1 (Kaltenbach et al., 2007). Interestingly the affinity of mHtt to IP₃R1 increases when mHtt is associated with HAP1a (Tang et al., 2003). Moreover, mHtt, but not normal Htt, augmented IP₃R1 activity in planar lipid bilayers (Tang et al., 2003). Similarly, application of subthreshold concentrations of DHPG, an mGluR1/5 agonist, sensitized Ca²⁺ release in YAC128 primary MSN cultures (Tang et al., 2003). This is consistent with the fact that glutamate-induced apoptosis of MSNs from YAC128 mice is mediated by mGluR1/5 and NR2B receptors (Tang et al., 2005). In fact, specific blockade of IP₃R1 with 2-APB and Enoxaparin is neuroprotective in the same model (Tang et al., 2005). In a follow-up study, we found disturbed Ca²⁺ signaling, enhanced glutamate-induced apoptosis, and augmented NR2B activity in cultured MSNs from YAC128 mice, which express full-length human Htt, but these effects were completely absent in shortstop mice that express an amino-terminal fragment of mHtt (exons 1 and 2), suggesting a critical role for full-length mHtt in altered MSN Ca²⁺ signaling (Zhang et al., 2008). In more recent experiments, it was demonstrated that viral delivery of a peptide that disrupts mHtt association with IP₃R1 protected YAC128 MSNs *in vitro* and *in vivo* (Tang et al., 2009). Augmented IP₃R1 activity further implicates mGluR5 receptor signaling in HD pathology. Importantly, inhibitors of IP₃R1 may impede intracellular Ca²⁺ overload early in the disease state and protect MSNs from glutamate-induced excitotoxicity. MSN are highly enriched for mGluR5, a member of the group I mGluRs (Testa et al., 1995; Kerner et al., 1997; Tallaksen-Greene et al., 1998; Mao and Wang, 2001, 2002). Stimulation of group I mGluR in MSN leads to the generation of IP₃ and release of Ca²⁺. The alterations in ER enzymes that have been observed in HD postmortem brains (Cross et al., 1985) are consistent with malfunction of ER Ca²⁺ handling in HD MSN neurons.

III. Mutant Huntingtin activates NR2B-containing NMDA receptors

MSN abundantly express NR2B subtype of NMDA receptors (Monyer et al., 1994; Landwehrmeyer et al., 1995; Portera-Cailliau et al., 1996). In contrast to NMDA receptors containing NR2A subtype, NR2B-containing NMDA receptors have significant permeability for Ca²⁺ and activation of these receptors may have a dramatic effect on intracellular Ca²⁺ signals in MSN. Importantly, studies from Lynn Raymond's and Michael Hayden's laboratories suggested that expression of mutant Htt^{exp} protein facilitates activity of NR2B subtype of NMDAR receptors in a heterologous HEK293 cells expression system (Chen et

al., 1999). Interestingly, the potentiating effect of Htt^{exp} was specific for the NR1/NR2B NMDAR subtype and not for the NR1/NR2A NMDAR subtype. Using the same HEK293 cells expression system it was also demonstrated that cells co-transfected with NMDAR and Htt-138Q plasmids were more sensitive to NMDA-induced apoptosis than the cells co-transfected with NMDAR and Htt-15Q or GFP (control) plasmids (Zeron et al., 2001). Similar to effects on NMDAR currents, potentiating effects of Htt-138Q on excitotoxic cell death were more pronounced in the presence of the NR1/NR2B NMDAR subunit combination than in the presence of the NR1/NR2A subunit combination (Zeron et al., 2001). These initial findings were corroborated in the YAC mouse, whereby facilitation of NMDA-evoked current amplitude in MSNs from YAC72 and YAC128 mice compared to WT was induced by the NR1/NR2B receptor (Zeron et al., 2002; Zhang et al., 2008). Similarly, cultured MSNs from YAC72 mice showed enhanced NMDA-mediated cell death relative to WT. Furthermore, treatment with ifenprodil, an NR2B-type selective antagonist, blocked most NMDA-mediated cell death in both YAC72 and WT mice (Zeron et al., 2002). The neuroprotective effect of ifenprodil was later recapitulated in YAC128 MSN cultures (Tang et al., 2005). Findings from dissociated MSNs from presymptomatic R6/2 mice have revealed that NR2A, and not NR1 or NR2B, mRNA levels are reduced, which is concomitant with larger NR2B-mediated NMDAR currents compared to WT (Ali and Levine, 2006).

The studies that indicated NR2A-type NMDARs are targeted primarily to the synapse and NR2B-type receptors are preferentially expressed at extrasynaptic sites (outside the synapse) is intriguing (Li et al., 1998; Stocca and Vicini, 1998; Tovar and Westbrook, 1999). In fact, dual roles for synaptic and extrasynaptic NMDARs have been proposed (for details see (Vanhoutte and Bading, 2003; Papadia and Hardingham, 2007)). Whereas synaptic NMDARs activate pro-survival pathways (e.g., CREB phosphorylation, BDNF transcription, ERK activation, antioxidant mechanisms), extrasynaptic NMDARs activate pathways mediate cell death (e.g., loss of mitochondrial membrane potential, inhibition of ERK and BDNF, dendritic blebbing) (Hardingham et al., 2002; Papadia et al., 2008; Goux et al., 2009). A recent study by Milnerwood and colleagues has shown that, indeed, mHtt results in increased extrasynaptic NMDAR expression and currents (Milnerwood et al., 2010) (also see (Li et al., 2004)). Examination of extrasynaptic activation, by mimicking glutamate spillover into the synaptic cleft with either strong evoked excitatory postsynaptic currents (EPSCs) or paired-pulse stimuli, revealed increased EPSC charge and elevated NMDA peak current and charge in presymptomatic (1 month) YAC128 MSNs relative to YAC18 (Milnerwood et al., 2010). Accordingly, application of TBOA, a GLT1 transport inhibitor that enhances glutamate spillover to extrasynaptic receptors, resulted in slowing of NMDAR EPSC kinetics in WT and YAC18 mice, but the slowing was consistently more augmented in YAC72 and YAC128. Moreover, in the presence of TBOA, NMDA currents and peak charge were larger in YAC128 compared to WT and YAC18, while YAC72 exhibited intermediate values. Altered TBOA-induced NMDA currents in YAC128 mice were ameliorated upon application of ifenprodil, which blocks NR2B extrasynaptic signaling. Spontaneous NMDA activity was not altered by mHtt, further suggesting involvement of NR2B-type and not NR2A-type NMDARs in altered MSN function. Surprisingly, treatment with low dose memantine, which is thought to preferentially antagonize extrasynaptic over synaptic

NMDARs, in 2-4 month YAC128 mice resulted in increased CREB activation and improved motor learning (number of falls when learning the rotarod) relative to WT mice (Milnerwood et al., 2010). Direct *in vivo* evidence for NR2B-mediated excitotoxicity in HD comes from recent findings demonstrating exacerbated striatal neurodegeneration in double-mutant mice that express 150Q and overexpress NR2B subunits (Heng et al., 2009).

How does mHtt augment NR2B activity? Numerous studies have investigated mRNA and protein expression of NMDARs in human postmortem brain and in HD mouse models, yet no conclusive correlation exists between CAG repeat number and receptor expression levels, nor receptor sensitivity (for discussion see (Milnerwood, 2006)). Some studies suggested that Htt^{exp} affects NMDAR function via PSD95-dependent mechanism (Sun et al., 2001; Song et al., 2003). Indeed, treatment of YAC72 and YAC128 MSN cultures with the Tat-NR2B9C peptide (200 nM), which blocks binding of NR2B with PSD95 and reduces surface expression of NMDARs, resulted in a marked reduction in NMDA-induced apoptosis (Fan et al., 2009). Knockdown of PSD95 with siRNA resulted in the same effect in YAC128 MSNs (Fan et al., 2009). Another possibility is related to changes in NMDAR trafficking to the plasma membrane in the presence of Htt^{exp} mutation (Fan et al., 2007). Future experiments will be needed to clarify exact molecular mechanisms responsible for increase in NMDAR activity in HD MSNs.

From above discussion is clear that hyperfunction of extrasynaptic NMDARs (NR2B) is a major problem in HD and these receptors warrant targeting. The NR2B-specific inhibitors (e.g., ifenprodil, memantine) are likely candidate targets. Indeed, low dose memantine is neuroprotective *in vitro* (Wu et al., 2006a; Okamoto et al., 2009a) and *in vivo* (Okamoto et al., 2009a; Milnerwood et al., 2010). Memantine demonstrated some beneficial effects in small scale pilot evaluation in HD patients (Ondo et al., 2007) and large phase IV clinical trial of memantine in HD patients is currently in progress. Furthermore, a continued search for compounds that positively regulate synaptic NMDARs (NR2A) and/or negatively regulate extrasynaptic NMDARs (NR2B) is warranted.

IV. Deranged Ca²⁺ signaling and apoptosis of HD MSN

Several lines of evidence indicate that glutamate-mediated excitotoxicity plays a role in neurodegeneration of HD MSN. Striatal injection of kainic acid induced death of MSN and yielded one of the first animal models of HD (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). Importantly, effects of kainate required presence of corticostriatal neurons (McGeer et al., 1978), suggesting that glutamate release is required for kainate-induced MSN cell death. More direct evidence for an involvement of NMDAR was obtained when HD-like lesions were observed following striatal injection of the NMDAR agonist quinolinic acid (Beal et al., 1986; Hantraye et al., 1990; Beal et al., 1991). Consistent with the excitotoxicity hypothesis, striatal neurons from YAC72 mouse were sensitized to neuronal death induced by quinolinic acid and NMDA (Zeron et al., 2002). Moreover, excitotoxic cell death of YAC72 MSN was blocked by ifenprodil (Zeron et al., 2002), supporting a direct involvement of NR1/NR2B NMDAR subtypes in HD.

Based on the results described above (sections II and III) we previously suggested that overactivation of IP₃R1-mediated Ca²⁺ release and NR2B-mediated Ca²⁺ influx in HD MSN may lead to Ca²⁺ overload and apoptosis of these neurons (Bezprozvanny and Hayden, 2004). To test this “Ca²⁺ hypothesis of HD” my laboratory used TUNEL assay to compare glutamate-induced apoptosis of MSN cultured from wild type mice and mice expressing mutant human Htt-128Q gene (YAC128 mouse (Slow et al., 2003)). The mice expressing normal human Htt-18Q gene (YAC18 (Hodgson et al., 1999)) was used as a control in these experiments. At 14 DIV all 3 groups of MSN were challenged by an 8 h application of glutamate (from 0 to 250 μM) to mimic physiological stimulation. Following exposure to glutamate, MSN were fixed, permeabilized and scored for apoptotic cell death using TUNEL staining. We determined that in basal conditions (no glutamate added) approximately 10% of MSN in all 3 experimental groups were apoptotic (TUNEL-positive). Addition of 25 μM or 50 μM glutamate increased the number of apoptotic cells to 15-20% in all 3 experimental groups. Addition of 100 μM or 250 μM glutamate increased apoptotic death to 60-70% for YAC128 MSN, but only to 25-30% for wild type and YAC18 MSN. Thus we reasoned that exposure to glutamate concentrations in 100 – 250 μM range leads to selective apoptosis of YAC128 MSN (Tang et al., 2005).

This “*in vitro* HD” model enabled us to test a connection between abnormal Ca²⁺ signaling and apoptosis of HD MSN. We found that inhibition of mGluR1/5 receptors (by a mixture of MPEP and CPCCOEt) reduced the glutamate-induced apoptosis of YAC128 MSN to WT MSN levels. NMDAR-inhibitor (+) MK801 or NR2B-specific antagonist ifenprodil had similar neuroprotective effects. Consistent with direct involvement of IP₃R1, preincubation of the MSN cultures with a membrane-permeable IP₃R blocker 2-APB (Maruyama et al., 1997) protected YAC128 MSN from glutamate-induced apoptosis. All these results supported an idea that glutamate-induced Ca²⁺ overload plays a key role in induction of apoptotic cell death of HD MSN. In more recent experiments, we demonstrated that viral delivery of a peptide that disrupts mHtt association with IP₃R1 protected YAC128 MSNs in vitro and in vivo (Tang et al., 2009).

V. IP₃R and abnormal Ca²⁺ signaling in SCA2 neurons

SCA2 patients suffer from a progressive cerebellar syndrome with ataxia of gait and stance, ataxia of limb movements, and dysarthria (Filla et al., 1999; Schols et al., 2004; Lastres-Becker et al., 2008). Similar to HD, the SCA2 is caused by an expansion and translation of unstable CAG repeats in the gene encoding ataxin-2 from the normal 22 to more than 31 extra glutamine repeats (Imbert et al., 1996; Pulst et al., 1996; Sanpei et al., 1996). The pathogenesis of SCA2 is currently not understood. Similar to wildtype ataxin-2, polyglutamine-expanded ataxin-2 protein is ubiquitously expressed in cells without severe aggregation and formation of inclusion bodies (Huynh et al., 2000), but the cerebellar PCs in SCA2 patients are mostly affected with a loss of over 75% (Schols et al., 2004). Genetic knockouts of ATXN2 orthologs in fly and worm resulted in embryonic lethality (Kiehl et al., 2000; Satterfield et al., 2002). Atxn2 knockout mice were viable but displayed a late-onset obesity phenotype (Kiehl et al., 2006). Mice deficient in Atxn2 did not show Purkinje cell loss or marked changes in the Purkinje cell dendritic tree (Kiehl et al., 2006). Non-essential role of Atxn2 in rodents is most likely related to the presence of orthologs and redundancy in

its function (Kiehl et al., 2006). Thus, the polyglutamine expansion in ataxin-2 likely does not cause a loss of function nor a dominant negative effect but a gain of toxic function (Kiehl et al., 2006). The role of calcium signaling in the pathogenesis of SCA2 is supported by the genetic association between polymorphisms in the CACNA1A gene and the age of disease onset in patients diagnosed with SCA2. The CACNA1A gene encodes the pore-forming $\alpha 1A$ subunit of $Ca_v2.1$, a P/Q-type voltage-gated calcium channel. Patients with a prematurely early age of onset of SCA2 tended to have a longer CAG-repeat length in the CACNA1A gene (Pulst et al., 2005). Longer CAG-repeat lengths in this gene have been genetically linked to SCA6.

The role of aberrant neuronal calcium signaling in SCA2 pathogenesis was strengthened further by a finding in our lab that $ATX2^{exp}$ but not wildtype $ATX2$ ($ATX2^{wt}$) specifically binds IP_3R1 (Liu et al., 2009). This suggested that its association would result in either a sensitization or desensitization of IP_3R1 to activation by inositol 1,4,5-triphosphate (IP_3) during glutamate signaling in PCs. PCs express extremely high levels of intracellular IP_3R1 (Furuichi et al., 1993; Sharp et al., 1999), which are present on endoplasmic reticulum (ER) membranes. $ATX2^{exp}$ and $ATX2^{wt}$ were found to localize and associate with ER membranes (van de Loo et al., 2009). In a lipid bilayer reconstitution experiment, we examined the effect of $ATX2^{exp}$ expression on IP_3R1 activation in single channel recordings of IP_3R1 co-expressed with $ATX2^{exp}$. We found the presence of $ATX2^{exp}$ substantially sensitized IP_3R1 to activation by inositol 1,4,5 triphosphate (IP_3) (Liu et al., 2009).

To test the importance of Ca^{2+} signaling in SCA2 pathogenesis we performed a series of experiments with SCA2 transgenic mouse model (SCA2-58Q, generated by (Huynh et al., 2000)) expressing 58 glutamine repeats ($ATX2^{exp}$) in the ataxin-2 gene under the control of the L7/Pcp2 PC-specific promoter. These mice exhibit behavioral deficits, loss of Purkinje cell dendritic arborization and Purkinje cell death, which is progressive and akin to SCA2 patient pathology (Huynh et al., 2000). We found that there was a significant increase in calcium release from ER stores via $ITPR1$ s in calcium imaging experiments in primary PCs cultured from SCA2-58Q transgenic mice, which was not observed in PCs cultured from wildtype littermates (Liu et al., 2009). When ryanodine or dantrolene were added to block ryanodine receptors and ER calcium release in PC cultures, the effect of $ATX2^{exp}$ expression was immediately reversed as ER calcium release returned to wildtype levels (Liu et al., 2009). In a TUNEL assay of PC death, we found that the addition of dantrolene to block the excessive ER calcium release caused by $ATX2^{exp}$ expression attenuated exogenous glutamate-induced PC death (Liu et al., 2009). Furthermore, long-term feeding of SCA2-58Q mice with a calcium stabilizer dantrolene alleviated the age-dependent motor coordination deficits in these mice quantified by rotarod and beam-walk behavioral assays (Liu et al., 2009). Stereological counting of PCs showed a rescue of PC death in 12-month old SCA2-58Q mice fed with dantrolene (Liu et al., 2009). The above lines of evidence supported the hypothesis that deranged calcium signaling plays a role in SCA2 pathogenesis.

VI. IP_3R and abnormal Ca^{2+} signaling in SCA3 neurons

SCA3 is a multi systemic disorder characterized by degeneration of spinocerebellar tracts, dentate nucleus, pontine and other brainstem nuclei, substantia nigra and pallidum (Stevanin

et al., 2000). In contrast to most other SCA, the cerebellar cortex and the inferior olives are widely spared. MRIs show cerebellar atrophy, which is less pronounced than in SCA2 in combination with brainstem atrophy. Nuclear inclusions containing expanded ataxin-3 have been found in neurons of affected brain regions. The clinical picture of SCA3 is characterized by a wide range of clinical manifestations, the precise nature of which partly depends on repeat length. All SCA3 patients suffer from a progressive syndrome with ataxia of gait and stance, ataxia of limb movements, and dysarthria (Coutinho and Andrade, 1978). At molecular level the cause of SCA3 is a polyQ expansion in the carboxy-terminal of ataxin-3 (Atxn3) protein (Paulson et al., 1997). The ataxin-3 is a 43 kDa cytosolic protein of 316 amino acids which contains amino-terminal Josephin domain and 3 ubiquitin-interactions motifs (UIMs) (Scheel et al., 2003; Albrecht et al., 2004). Consistent with the presence of Josephin domain Atxn3 functions as deubiquitinating enzyme (DUB) (Burnett et al., 2003). The polyQ-expansion has no effect on DUB activity of Atxn3 (Burnett et al., 2003; Winborn et al., 2008), so it is not likely that neurodegeneration in SCA3 develops due to impaired normal function of Atxn3. Ataxin-3 has been shown to act as a potent transcriptional repressor, a function that is probably related to its DUB activity (Evert et al., 2006). Knockout of the *ATXN3* gene in mice increases protein ubiquitination but does not result in an obvious behavioral phenotype (Schmitt et al., 2007).

Abnormal neuronal Ca^{2+} signaling has also been implicated in SCA3 pathogenesis. It was discovered that inhibition of Ca^{2+} -dependent protease calpain suppressed aggregation of polyglutamine-expanded Atxn3 in transfected cells (Haacke et al., 2007). Similar to mutant Htt and mutant Atxn2, mutant Atxn3 but not wild type Atxn3 binds to and activates $\text{IP}_3\text{R1}$, an intracellular Ca^{2+} release channel (Chen et al., 2008). Moreover, long-term feeding of SCA3-YAC-84Q transgenic mouse with Ca^{2+} stabilizer dantrolene alleviated age-dependent motor coordination deficits in this mice and prevented neuronal loss in SNc and PN (Chen et al., 2008). These results provided strong experimental support to the “ Ca^{2+} hypothesis of SCA3 pathogenesis” (Chen et al., 2008).

Abnormal neuronal Ca^{2+} signaling is not restricted to polyglutamine-expansion ataxias and may be important in other ataxias as well. For example, recent genetic evidence indicated that the cause of SCA15 is deletion of a fragment of a gene encoding $\text{IP}_3\text{R1}$ (van de Leemput et al., 2007).

VII. Ca^{2+} blockers and perspectives for clinical intervention in HD and SCA patients

Despite efforts by many laboratories and cloning of Huntingtin and ataxins more than 15 years ago, there is still no cure for these disorders. The most obvious targets are mutant Huntingtin, Atxn2 and Atxn3 proteins themselves. Expression of mutant Htt and mutant ataxin-1 (Atxn1) was reduced by injection of RNAi-encoding viruses, resulting in beneficial effects in mouse models of HD and SCA1 (Xia et al., 2004; Harper et al., 2005). Similar RNAi knockdown approach could also be expanded to SCA2 and SCA3. Selective knockdown of mRNA encoding mutant Htt and mutant Atxn3 proteins was recently demonstrated using peptide nucleic acid (PNA) and locked nucleic acid (LNA) antisense

oligomers targeting expanded CAG repeats (Hu et al., 2009). These are very attractive approaches, but their utility at the moment is limited by the absence of RNAi or antisense brain delivery system that can be used in humans.

In addition to reducing expression of polyQ-expanded proteins at the mRNA level, another potential therapeutic strategy is based on developing agents that selectively bind to mutant forms of these proteins. A polyQ-containing protein can exist in multiple conformations, some of which are nontoxic and some of which are aggregation-prone and toxic (Shao and Diamond, 2007). One possibility to reduce the amount of toxic conformations of Atxn2 and Atxn3 proteins is to increase the levels of chaperons, which control protein folding. Although these approaches are being developed for polyQ-expansion disorders (Warrick et al., 1999; Wytenbach et al., 2002; Wacker et al., 2004), they have not produced clinical leads yet. Several small molecules were isolated in screens as inhibitors of polyQ aggregation. Some of these molecules were able to reduce polyQ aggregation and toxicity in cellular and animal models (Heiser et al., 2002; Sanchez et al., 2003; Wang et al., 2005; Zhang et al., 2005; Ehrnhoefer et al., 2006; Chopra et al., 2007). A specific polyQ binding peptide (QBP1) was isolated in the phage library screen (Nagai et al., 2000). It has been reported that QBP1 peptide prevented toxic conformational transition within polyQ-expanded proteins (Nagai et al., 2000) and exerted neuroprotective effects in cellular and animal models of polyQ toxicity (Nagai et al., 2003; Popiel et al., 2007, 2009). Despite excellent scientific rationals, so far none of these candidates has successfully advanced into clinical trials due to problems related to finding an appropriate mode of delivery, poor pharmacokinetics and low efficacy *in vivo*. Recent solution of the crystal structure of Htt exon 1 containing polyQ region (Kim et al., 2009) opens perspective for rational design of novel polyQ-binding therapeutic agents.

Our laboratory demonstrated beneficial effects of Ca^{2+} stabilizer dantrolene in both SCA2 and SCA3 mouse models (Chen et al., 2008; Liu et al., 2009). Dantrolene also has a long history of human use for treatment of malignant hyperthermia and for neurological indications. In considering design of the clinical trial potential side effects of long term exposure to lithium or dantrolene must be taken into consideration. NMDAR inhibitor memantine and anti-glutamate agent riluzole were neuroprotective in experiments with primary MSN cultures from YAC128 HD mouse model, with memantine being more effective (Wu et al., 2006b). Memantine was also effective in 3-NP model of HD (Lee et al., 2006) and in YAC128 genetic HD model (Okamoto et al., 2009b). Memantine demonstrated some beneficial effects in small scale pilot evaluation in HD patients (Ondo et al., 2007) and is currently being tested soon in phase IV HD clinical trial. Riluzole was tested in phase III HD clinical trial, but it was not successful (Landwehrmeyer et al., 2007). In addition to NMDAR, voltage-gated Ca^{2+} channels constitute another potential target for SCA treatment. In recent studies L-type Ca^{2+} channel inhibitor isradipine significantly protected SNc neurons in animal models of Parkinson's disease (Chan et al., 2007). Moreover, a recent retrospective epidemiological study has found that treatment of hypertension with Ca^{2+} channel antagonists significantly diminished the risk of developing PD (Becker et al., 2008). It is possible that isradipine and other dihydropyridines (DHP) may also be useful for treatment of HD, SCA2 and SCA3 patients. More conservative potential strategy involves use of mitochondrial stabilizers and energizers, such as creatine, CoQ10, and MitoQ. In

previous neurodegenerative trials these compounds resulted in modest benefit (Chaturvedi and Beal, 2008) and it is likely that similar result will be obtained for SCA2 and SCA3 patients as well. Controlled clinical evaluation of Ca^{2+} inhibitors in HD, SCA2 and SCA3 patients will provide an ultimate test to the proposed “ Ca^{2+} hypothesis” of these disorders. .

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