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Research report

Analysis of cellular, transgenic and human models of Huntington's disease reveals tyrosine hydroxylase alterations and substantia nigra neuropathology

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Abstract

Huntington's disease (HD) is a progressive, autosomal dominant neurodegenerative disorder that is pathologically characterized by a striatal-specific degeneration. Aberrant dopamine neurotransmission has been proposed as a mechanism underlying the movement disorder of HD. We report that the enzymatic activity of tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine biosynthesis, is decreased in a transgenic mouse model of HD. In addition, mutant huntingtin was found to disrupt transcription of TH and dopamine β-hydroxylase (DβH) promoter reporter constructs. In situ hybridization revealed extensive loss of TH mRNA and decreased dopaminergic cell size in human HD substantia nigra. TH-immunoreactive protein was reduced in human grade 4 HD substantia nigra by 32% compared to age-matched controls. These findings implicate abnormalities in dopamine neurotransmission in HD and may provide new insights into targets for pharmacotherapy.
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Theme: Disorders of the nervous system
Topic: Degenerative disease: other

Keywords: Tyrosine hydroxylase; Huntingtin; Substantia nigra; Huntington's disease; Dopamine; mRNA

1. Introduction

Huntington's disease is a devastating neurodegenerative disorder with mid-life onset, caused by a trinucleotide CAG repeat in exon 1 of the human *HD* gene [20]. Although the huntingtin protein (Htt) is ubiquitously expressed throughout the brain, a regionally preferential neurodegeneration of medium spiny GABAergic neurons in the striatum occurs in HD. The exact function of wild-type Htt, as well as the pathogenic mechanism of the mutant protein, is unknown. However, recent studies have shown that normal Htt is important in a number of cellular processes such as the functioning of nuclear organelles [19], RNA biogenesis [14], hematopoiesis [29], and vesicular trafficking [46].

The first genetic mouse models of HD (R6/2 mice) were introduced in 1996 [28]. R6/2 mice express a portion (exon 1) of the disease-causing form of human Htt and develop a behavioral phenotype that suggests dysfunction of dopaminergic neurotransmission [3,6,39]. Initial characterization of these mice revealed a loss of dopamine receptors as early as 4 weeks of age, before the

Abbreviations: D1, dopamine receptor D1; D2, dopamine receptor D2; DβH, dopamine-β-hydroxylase; HD, Huntington's disease; Htt, huntingtin; HVA, homovanillic acid; L-DOPA, levo-dihydroxyphenylalanine; nt, nucleotides; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase

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onset of symptoms, paralleling changes observed in human HD [2,8,37]. Reductions in dopamine- and cAMP-regulated phosphoprotein (DARPP-32) in presymptomatic HD mice suggest that alterations to the D1 dopamine receptor/DARPP-32 signaling cascade may also contribute to HD pathogenesis [3]. Levels of dopamine and immunoreactive tyrosine hydroxylase (TH) are decreased in the striatum of R6/2 mice [1,36]. In fact, treatment of the R6/2 mice with L-DOPA to replace dopamine improves some motor abnormalities in R6/2 mice [18]. Recently, the size of TH-positive neurons in the substantia nigra has been found to be decreased in the human HD brain [31], as well as in 16-week-old R6/1 mice [33]. Similarly, post-mortem studies reveal atrophy of human nigrostriatal pathway [15]. To date, alterations in TH activity and mRNA have not been reported in either the genetic models or human cases of HD.

We hypothesized that a major reason for the dopaminergic changes that have been observed in HD is disruption of TH homeostasis. Tyrosine hydroxylase (TH; EC 1.14.16.2) is the rate-limiting enzyme in the biosynthetic pathway of the catecholamines: dopamine, norepinephrine and epinephrine. TH-containing cell bodies reside in the substantia nigra; loss of these cells has been widely implicated in the pathology of Parkinson’s disease. Although Htt is known to be expressed in human substantia nigra [23], involvement of the nigrostriatal pathway in HD remains unclear. We present data using cellular and transgenic mouse models of HD, as well as human HD samples, which suggest that the dopaminergic system is disrupted in HD. We observed large changes in TH activity, protein and mRNA in HD tissues. We also found that expression of mutant Htt inhibited TH and DβH promoter-mediated transcription in an inducible PC12 cell model of HD. Taken together, these findings demonstrate that the substantia nigra is a locus of cellular dysfunction in HD and suggests that restoration of TH activity may prove efficacious in the treatment of HD.

2. Materials and methods

2.1. Materials

All materials were obtained from Sigma (St. Louis, MO) with the following exceptions; L-[5-^{3}H] tyrosine and chemiluminescent reagents (Renaissance) were from DuPont NEN Research Products (Boston, MA); and activated charcoal (Darco G-60) was from Fisher Scientific (Pittsburgh, PA). PVDF and Kaleidoscope pre-stained protein ladder were purchased from Bio-Rad (Hercules, CA). Mouse anti-TH monoclonal antibody (TH-2) was obtained from Sigma. Mouse anti-actin monoclonal antibody was purchased from Chemicon International (Temecula, CA). Sheep anti-mouse IgG HRP-coupled secondary antibody was purchased from Amersham Life Sciences (Arlington Heights, IL).

2.2. Transgenic animals and human HD brain tissues

Transgenic mice that contain exon 1 of the human huntingtin gene (R6/2) and age-matched littermate control mice were sacrificed by decapitation at 4, 8 and 12 weeks of age. Brains were removed and the brain stem was dissected, weighed, and stored at −80 °C until TH activity and protein analyses could be performed. Brains were kindly provided by Dr. Gillian Bates (GKT School of Medicine, London, UK). Grade 4 human HD and non-disease control midbrains were provided by the Harvard Brain Tissue Resource Center. The brains were cut in 12-μm sections for in situ hybridization studies (see Table 1 for subject information).

2.3. Radioenzymatic TH activity assay

A radioenzymatic activity assay for TH was utilized on 25 μl aliquots of striatal homogenates from 4-, 8- and 12-week-old R6/2 mice, as detailed by Reinhard et al. [34]. Each animal was assayed in duplicate and activity was normalized to the amount of total protein in the striatal homogenate (nmol/h/μg). Six wild-type and R6/2 mice at each time point were analyzed for TH activity, except at 4 weeks, when five wild-type mice were used.

2.4. TH Western analyses

TH-immunoreactive protein was measured with an anti-TH polyclonal antibody (TH-2) at 1:1000 with 50 μg of total protein as determined by protein assay [4]. The protein was resolved on a 12.5% acrylamide gel (Bio-Rad), transferred to PVDF for 1 h at 400 mA. Blots were blocked in 5% dry milk/Tris-buffered saline with Tween-20 (TBS-T) for 1 h and then exposed to a 1:1000 dilution of TH-2 overnight at 4 °C. All blots were then subjected to a goat anti-mouse-HRP antibody (1:3000), rinsed and visualized with chemiluminescent reagent (Renaissance™).

Table 1
Human postmortem substantia nigra samples

Sample	Age	Sex	PMI	HBTRC #
Control	50	M	26.50	4739
Control	53	M	20.18	4744
Control	56	F	11.83	4881
Control	56	M	20.88	4890
Control	74	F	12.17	5113
HD (grade 4)	74	F	15.80	4048
HD (grade 4)	56	F	9.50	4781
HD (grade 4)	49	M	16.60	4826
HD (grade 4)	52	M	18.16	4828
HD (grade 4)	52	M	22.17	5136

Substantia nigra from five grade 4 HD and age-matched controls were used to investigate whether TH mRNA levels and dopaminergic cell size are altered in HD. Postmortem interval (PMI) is represented as hours. Harvard Brain Tissue Resource Center numbers (HBTRC #) for the human brains are shown.

All blots were stripped with Western blot stripping buffer (Pierce Chemical, Rockford, IL), re-blocked in 5% milk in TBS-T and probed for actin immunoreactivity to verify equal protein loading.

2.5. In situ hybridization and emulsion staining

Radioactive in situ hybridization using an RNA probe for human TH (bp 308–698) was performed on five grade 4 HD cases and five age-matched control cases as previously described [11,41]. Samples were then subjected to emulsion autoradiography. Emulsion autoradiography provides a means of determining cellular mRNA expression within different brain regions. Slides were dipped in Ilford K5 emulsion (Polysciences, Warrington, PA) diluted 1:1 with distilled water, dried overnight, stored at 4 °C for 4 weeks and developed. Cell nuclei were visualized by counterstaining with hematoxylin and eosin. Quantitative microscopic analysis of hybridization intensity was performed using a computer-assisted image analysis system (M1; Imaging Research, St. Catharine's, Ontario, Canada).

Emulsion autoradiographic analysis was performed according to a method previously used by this laboratory [24,45]. In brief, neuromelanin-positive neurons of substantia nigra were visualized using bright-field optics under a 100 × water-immersion lens (Leitz). The image analysis system was used to outline the soma of each labeled neuron and quantify the overlying emulsion grains. The area of the neuronal profile (μm^2) and the number of silver grains present were recorded and used in order to compute the intensity of each neuron in grains per 1000 μm^2. The intensity of the autoradiographic signal was measured using a computer drawing tool, which outlines the cell body. Background signal was evaluated by encircling an equal number of similarly sized areas of white matter in the crus cerebri and quantifying grains within that area as a comparison to cell body grain clusters.

2.6. Cell culture, transfections and reporter gene assays

Parental, HD25Q and HD103Q PC12 cells were grown in Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Rockville, MD) supplemented with 10% horse serum, 5% fetal calf serum (FCS) and 1% penicillin/streptomycin. These cells contain exon 1 of the human *HD* gene with either 25 or 103 polyglutamines (25Q or 103Q; kindly supplied by Dr. Erik Schweitzer, University of California, Los Angeles), within the ecdysone-sensitive plasmid (Invitrogen, Carlsbad, CA). Expression of Htt exon 1 is inducible upon the addition of ponasterone A to the culture medium. Transfection was performed with Lipofectamine 2000 (Invitrogen). PC12 cells were grown in six-well plates and co-transfected with 1.5 μg of TH-luc or DβH-luc reporter plasmid (kind gift of Dr. Kwang-Soo Kim, McLean Hospital, Belmont, MA), as well as 2 μg of CMV-βgal for normalization of transfection efficiency. Cells were transfected in Opti-MEM medium for 5 h. Then, the cells were re-fed with DMEM with serum and allowed to grow overnight at 37 °C. The cells were then differentiated in DMEM (1% FCS) by adding nerve growth factor (1 × NGF). Htt expression was achieved by adding 1 μM ponasterone A (Invitrogen) and incubating for 18–24 h at 37 °C. Cells were rinsed with phosphate-buffered saline (PBS) and lysed in 1 × passive lysis buffer. Transcriptional activity of the TH and DβH promoters was measured in 20 μl of each cell lysate using a luciferase assay system (Promega). Three independent transfections and assays were performed.

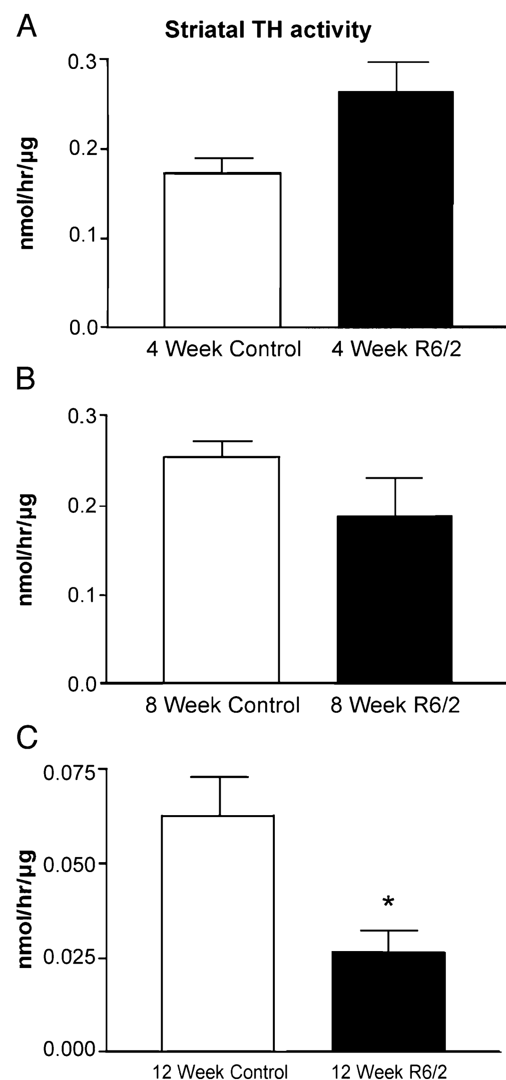

Fig. 1. Altered tyrosine hydroxylase activity in transgenic HD mice and in an inducible HD cell model. (A) Striatal TH activity in 4-week-old R6/2 mice. TH activity is increased 53% in the R6/2 mice ($p = 0.053$). (B) Striatal TH activity in 8-week-old R6/2 mice. A nonsignificant decrease in TH activity was observed in the R6/2 mice. (C) Striatal TH activity in 12-week-old R6/2 mice. TH activity is significantly decreased by 44% ($p = 0.03$). TH activity is represented as nmol/h/μg of total protein.

2.7. GST pull-down assay

A plasmid containing a full-length human TH cDNA (pET-21c-hTH) was in vitro translated with ^{35}S-methionine and incubated with equal amounts of Htt exon 1 constructs with 20, 32 and 53 polyglutamines (GST-CAG20Q, GST-CAG32Q and GST-CAG53Q, kindly supplied by Dr. Erich Wanker, Max Planck Institute, Berlin). GST-fusion proteins were produced and a GST pull-down assay was performed as previously described [48].

2.8. Image analysis and statistics

TH activity and protein level changes, as well as differences in TH and DβH promoter activities, were analyzed using unpaired Student's *t*-tests. Cellular mRNA expression levels assessed from emulsion autoradiograms were analyzed using an ANOVA with repeated measures design and Fisher's PLSD post hoc pairwise comparison. Statistical significance was associated with values of $p < 0.05$.

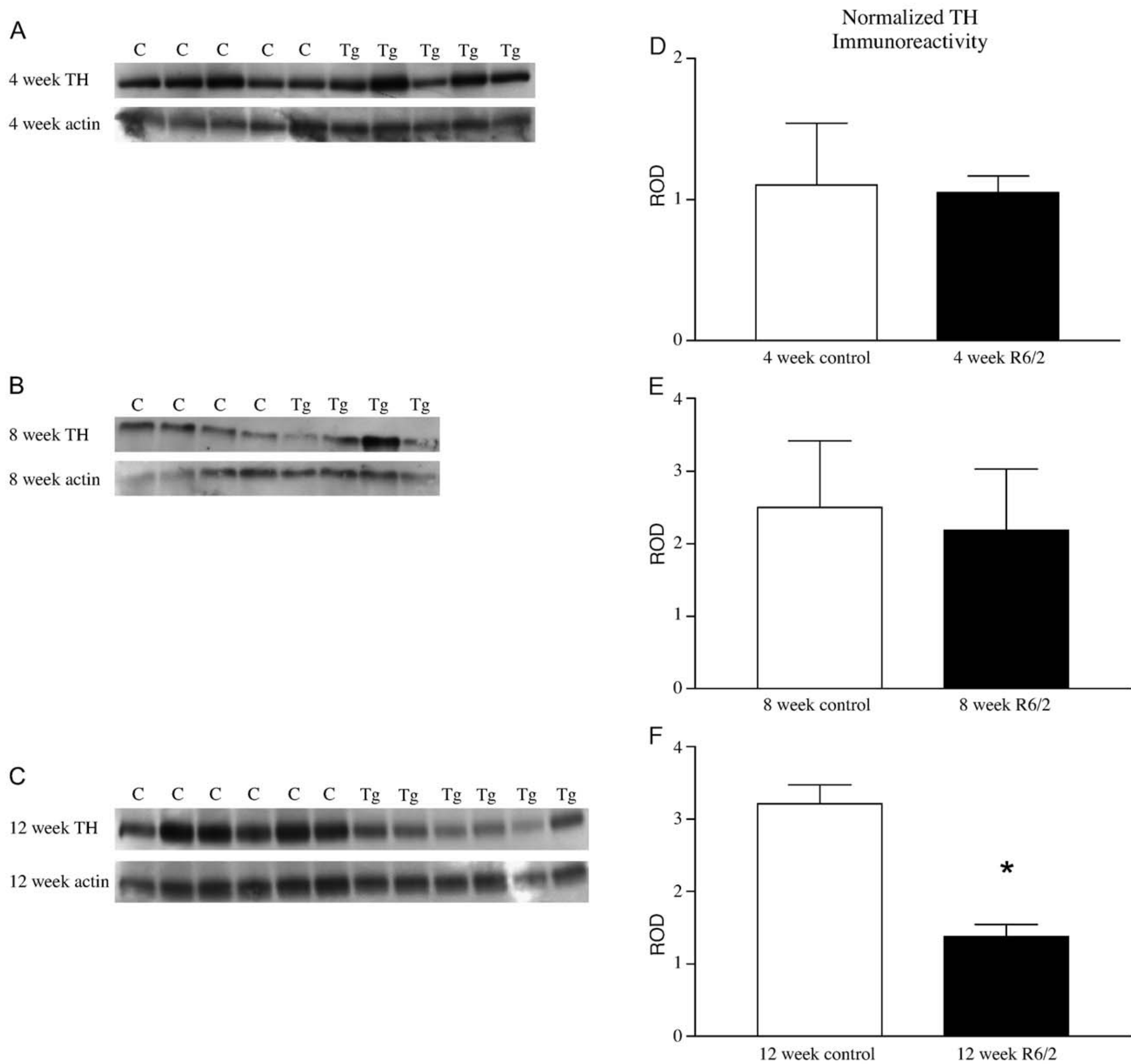

Fig. 2. TH protein is decreased in 12-week-old R6/2 mice. (A) 50 μg of total striatal protein from 4-week-old R6/2 and control mice. (B) 50 μg of total striatal protein from 8-week-old R6/2 and control mice. (C) 50 μg of total striatal protein from 12-week-old R6/2 and control mice. TH was visualized with an anti-TH monoclonal antibody (TH-2) at 1:2000 in TBS-T. Densitometric analysis of TH protein. (D) Relative optical densitometry for TH immunoreactivity in the 4-week-old R6/2 was unchanged when compared to age-matched controls. Immunoreactive signal was corrected with b-actin. (E) Relative optical densitometry for TH immunoreactivity in the 8-week-old R6/2 was unchanged when compared to age-matched controls. (F) Relative optical densitometry for TH immunoreactivity in the 12-week-old R6/2 was decreased by 57% when compared to age-matched controls ($p < 0.0001$).

3. Results

3.1. TH activity and protein in cellular and mouse models of HD

We hypothesized that TH inhibition in HD accounts for the diminished levels of dopamine and its metabolites that have been observed in both transgenic mice and human HD brain [18,35,36]. We therefore assayed tyrosine hydroxylase activity in the striata of 4-, 8- and 12-week-old R6/2 mice, as well as grade 4 human HD substantia nigra. In R6/2 mice, TH activity was found to be biphasic. At 4 weeks of age, TH activity was increased in the R6/2 mice (Fig. 1A; $p=0.053$) while protein levels remained unchanged (Fig. 2A and D). At 8 weeks of age, TH activity (Fig. 1B) and protein (Fig. 2B and E) were unchanged when compared to the wild-type littermate controls. However, at 12 weeks of age, both TH activity (Fig. 1C) and protein (Fig. 2C and F)

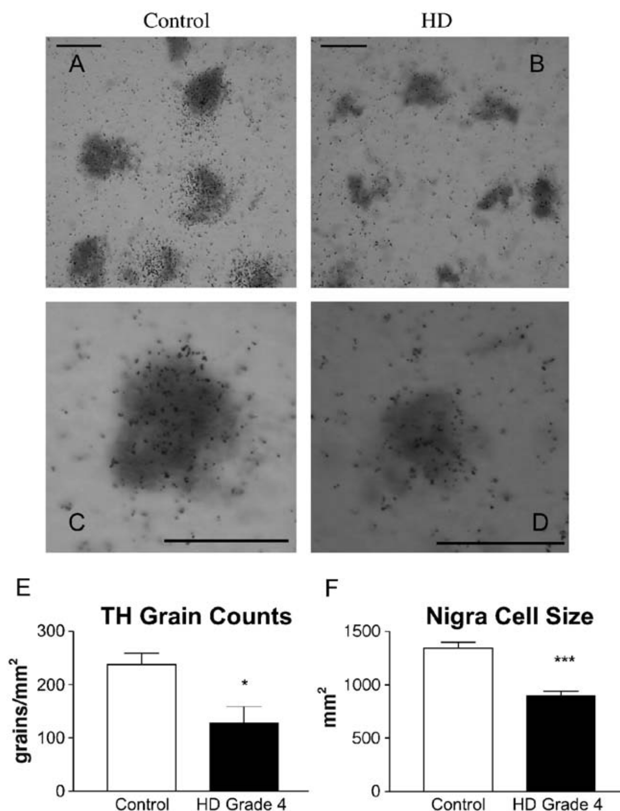

Fig. 3. Decreased mRNA for tyrosine hydroxylase in human HD substantia nigra. Radioactive in situ hybridization was performed with a probe specific for human TH. Small black dots represent exposed silver grains. Brown color is neuromelanin contained with SN neurons. (A) 40× control. (B) 40× HD. (C) 100× control. (D) 100× HD. (E) Silver grain counts in substantia nigra. Evidenced here is a reduction in silver grain intensity corresponding to decreased TH mRNA expression per cell. TH mRNA was reduced 46% in grade 4 HD compared to age-matched control subjects ($p=0.02$). (F) Substantia nigra cell size. The size of SN neurons is reduced 33% in the grade 4 HD cases when compared to the control cases ($p=0.0002$). Bar = 30 μm.

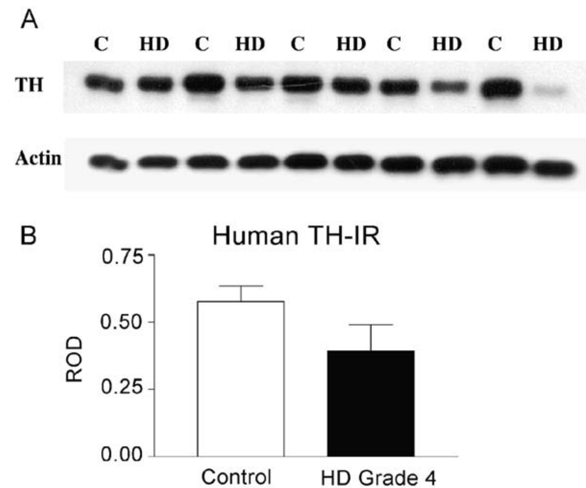

Fig. 4. TH protein is nonsignificantly decreased in human HD substantia nigra. (A) TH immunoreactivity in 50 μg of total nigral protein from grade 4 HD and control brains. Top panel (TH; 61 kDa) and lower panel (Actin; 43 kDa). Densitometric analysis of TH protein. (B) Relative optical densitometry for TH protein in the grade 4 HD substantia nigra was decreased by 32% when compared to age-matched controls ($p=0.14$). TH signal was normalized to actin protein.

were decreased by 43% ($p=0.03$) and 57% ($p<0.0001$), respectively, when compared to age-matched controls. Actin immunoreactivity was not changed at any age.

An inducible PC12 cell model of HD was analyzed to see if induction of exon 1 of mutant Htt inhibited endogenous TH activity. However, following a 24-h expression of mutant Htt (103Q), TH activity was not inhibited compared to the uninduced HD103Q cells (data not shown). In addition, Western analyses indicated that the expression of Htt with 25Q or 103Q had no effect on TH protein levels in the PC12 cells (data not shown).

3.2. TH mRNA, protein and dopaminergic cell size decreases in substantia nigra

We next wanted to determine if TH mRNA levels are altered in grade 4 HD human brains. In situ hybridization for TH was performed on sections of midbrain containing substantia nigra from five grade 4 HD cases and five age-matched control subjects (Table 1). Following Ilford emulsion staining, TH mRNA was visualized with light microscopy. We report that TH mRNA was significantly decreased in HD substantia nigra (Fig. 3B and D) compared to controls (Fig. 3A and C). Quantification of TH-positive silver grains revealed a 46% decrease (238 vs. 128 grains/mm^2) in the HD samples (Fig. 3E; $p=0.019$). In addition, nigral cell size was significantly reduced in the HD samples (Fig. 3F; $p=0.0002$). Neuromelanin-containing cells were approximately 33% smaller in the substantia nigra of the grade 4 HD brains (1343 ± 58 vs. 905 ± 38 μm^2).

Protein samples (50 μg) from each of the five grade 4 HD substantia nigra and five age-matched controls were ana-

lyzed for changes in TH immunoreactivity. TH protein was nonsignificantly reduced by 32% in the HD brain samples following normalization to actin protein ($p=0.14$; Fig. 4A and B).

3.3. Mutant huntingtin expression inhibits the TH promoter

An inducible PC12 cell model of HD (generous gift of Dr. Erik Schweitzer, UCLA) was used to determine if the expression of Htt has deleterious effects on the transcriptional activity of the TH and dopamine β-hydroxylase (DβH) promoters. DβH is the enzyme responsible for the synthesis of norepinephrine from dopamine. PC12 cells containing exon 1 of the human *HD* gene containing either 25 or 103 polyglutamines were transfected with luciferase reporter constructs driven by either 2.6 kb of the TH promoter (TH-luc) or the first 978 nt of the DβH promoter (DβH-luc). Addition of the inducing agent, ponasterone A, had no effect on TH- or DβH-mediated transcription in the parental PC12 cell line that does not contain the human Htt fragment (Fig. 5A and B). Expression of Htt with 25Q decreased both TH- and DβH-mediated transcription (Fig. 5A and B). These changes were not statistically significant. Expression of mutant Htt (103Q) significantly inhibited TH-mediated transcription (Fig. 5A; $p=0.03$). The HD103Q cells also displayed a trend towards decreases in DβH-mediated transcription, which did not reach statistical significance ($p=0.09$).

Fluorescence immunocytochemistry for TH was performed on all three HD PC12 cell lines using a mouse anti-TH antibody (TH-2). Expression of mutant Htt had no effect on TH expression levels in any of the cell lines tested. In addition, the subcellular localization of TH was not changed upon Htt 25Q or 103Q expression, nor was TH sequestered into the nuclear, cytoplasmic or perinuclear inclusions that have been demonstrated in the HD103Q cells (data not shown).

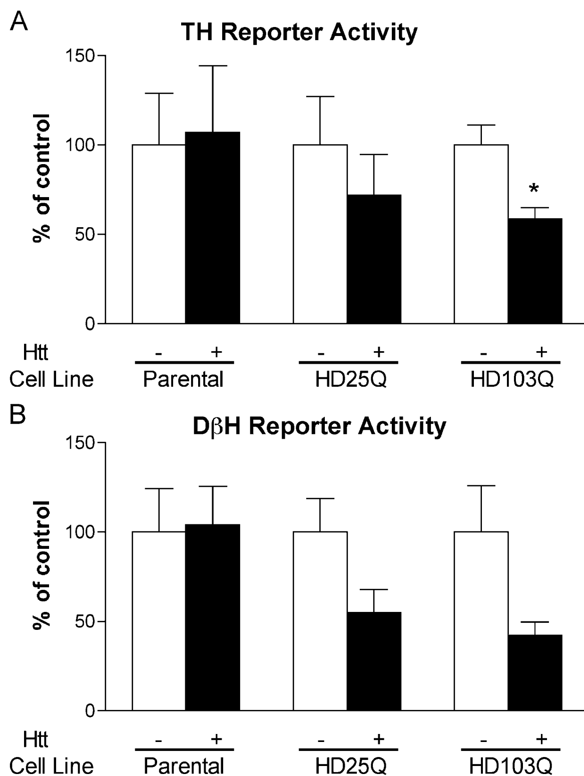

Fig. 5. TH and DβH reporter assays. (A) TH reporter assay. Addition of the inducing agent, ponasterone A (pon A), has no effect on either TH- or DβH-mediated transcription. (B) DβH reporter assay. Induction of normal glutamine length HD25Q protein with ponasterone A caused a nonsignificant repression of both TH- and DβH-mediated transcription ($p=0.47$ and $p=0.12$, respectively). Induction of the mutant HD103Q protein with ponasterone A caused a significant repression of TH-mediated transcription (*$p=0.03$). Nonsignificant repression of DβH-mediated transcription was observed upon HD103Q expression ($p=0.09$). Data represented are the mean ± S.E.M. of three independent experiments. Each experiment was assayed in triplicate for luciferase activity. β-Galactosidase activity was used to normalize luciferase data.

3.4. Human TH and exon 1 of Htt do not interact

Finally, we wanted to determine if TH and Htt interact in a polyglutamine-dependent manner. We used GST-fusion proteins of exon 1 of human Htt with 20, 32 or 53 polyglutamines (Gift of Dr. Erich Wanker). We have previously used these GST-fusion proteins in pull-down binding assays to demonstrate that GST-53Q interacts with thyroid hormone receptor (TRβ1) [49]. GST pull-down assays with a radiolabeled full-length human TH cDNA and the GST-Htt constructs showed that human TH and exon 1 of Htt do not interact in vitro (data not shown).

4. Discussion

These findings demonstrate significant alterations of TH activity and protein levels in the R6/2 transgenic mouse model and human HD substantia nigra. An early increase in TH activity (4 weeks) was followed by a significant inhibition of TH activity in the striata of 12-week-old R6/2 mice. Expression of mutant Htt in an inducible PC12 model of HD revealed that Htt significantly interferes with TH-promoter mediated transcription, but did not alter either TH activity or protein. In addition, deficits in TH protein, TH mRNA and neuromelanin cell atrophy in the substantia nigra of grade 4 human HD were also observed. Overall, these human data confirm that dopaminergic pathology occurs in HD.

A dopamine deficit in R6/2 mice has been previously reported [36]. It is likely that the inhibition of TH activity that we observed between 8 and 12 weeks of age accounts for the reduction in the catecholamines and their metabolites reported by Reynolds et al. We also assayed TH activity in the substantia nigra of human HD brain. However, we were unable to detect significant TH activity in

either the control or HD brains. This is likely the result of the postmortem interval and instability of TH enzymatic activity. Our GST pull-down assay results indicate that TH and exon 1 of Htt do not interact in vitro. Similarly, TH was not found to co-localize into Htt inclusions in the HD103Q PC12 cell line (data not shown). These results suggest that the inhibition of TH in the R6/2 mice is not due to an aberrant protein–protein interaction between mutant huntingtin and TH. It remains possible that the late-stage disruption of TH homeostasis (nigrostriatal pathway) observed in both the HD transgenic mouse and human HD cases are a response to the characteristic striatal-selective cell death of HD.

TH is a member of the amino acid hydroxylase superfamily of enzymes that includes tryptophan hydroxylase (TPH), the rate-limiting enzyme in the biosynthesis of serotonin. TH and TPH share approximately 50% amino acid homology and are thought to have evolved from a common progenitor [17]. Recently, our laboratory determined that TPH enzymatic activity is significantly inhibited in the R6/2 mice as early as 4 weeks of age [48]. Like TH, TPH does not bind to Htt in vitro. One possibility is that both TPH and TH are inhibited due to the increased levels of oxidative stress that have been demonstrated in this transgenic mouse line [44]. Recently, we have found that the transcription factor Sp1 exhibits increased binding to its DNA binding domain using electromobility shift assays in nuclear extracts from 12-week-old R6/2 mice when compared to age-matched controls. Increased Sp1 binding to DNA is often induced by oxidative stress [10]. Neuroprotective treatments might therefore restore TH and TPH activity in transgenic R6/2 mice.

It has also been proposed that the high concentration of dopamine in the basal ganglia may actually be neurotoxic and play an important role in the pathogenesis of HD [21]. Increased TH activity corresponds to increased cellular dopamine levels. Dopamine can auto-oxidize to form dopamine quinone, a reactive molecule which spontaneously decomposes to form additional reactive species that can have deleterious physiological effects [42]. This conversion may help explain why long-term treatment of the R6/2 mouse model of HD with dopamine precursor L-DOPA had long-term deleterious effects on both motor activity and survival [18]. Early observations in human HD samples by Spokes [40] support our theory that TH activation in the striatum may participate in the striatal specificity of HD. Spokes found significant increases in dopamine and norepinephrine concentrations in the striatum, nucleus accumbens and pars compacta of the substantia nigra of human HD brains [40]. It was originally thought that these increases in the cathecholamines indicated a sparing of the nigrostriatal pathway. However, if the increases in TH activity seen in the R6/2 mice also occur in the human disease, perhaps inhibiting TH activity in presymptomatic individuals would afford striatal neuroprotection. While unproven, this theory may also provide a clue to understanding the striatal-specific neurodegeneration that occurs in HD.

Dopamine receptor antagonists such as haloperidol have been used to manage both the chorea and psychosis that can occur in HD patients [38]. Past attempts to alter dopaminergic tone in HD patients with dopamine receptor agonists such as bromocriptine and apomorphine have yielded variable results [5,22]. In some patients, dopamine agonists produced transient symptomatic improvements, while other patients worsened. Dopamine autoreceptor agonists such as bromocriptine should decrease both the synthesis and release of dopamine. However, a problem with previous dopaminergic drugs is their lack of receptor specificity. It is conceivable that treatment of HD patients with newer highly selective D2/D3 receptor agonists currently used for Parkinson's disease, such as pramipexole or ropinirole, would be beneficial in slowing the progression or ameliorating the symptoms of HD.

Other postmortem HD neurochemical studies have suggested that dopaminergic homeostasis is altered in human HD. Dopamine levels in the caudate and putamen of postmortem HD brain were similar to those of age-matched controls [35,47]. However, both groups found that homovanillic acid (HVA) levels were significantly reduced in HD. One study of human postmortem HD substantia nigra revealed a 40% decrease in neuronal number and cellular atrophy of the pigmented and nonpigmented neurons [31]. We found a 33% decrease in the size of neuromelanin-containing neurons within the substantia nigra of HD cases, which is in agreement with earlier studies in the R6/1 mice that reported a 15% decrease in neuromelanin-containing cell size [33]. Dopamine receptor binding studies found that D1 and D2 receptor binding was decreased 45–50% in HD putamen [12]. D1 binding was similarly decreased in the substantia nigra pars reticulata, but unchanged in the substantia nigra pars compacta. Both the reduction in dopaminergic metabolites and D1 and D2 binding have also been observed in the R6/2 model of HD [3,8,9,36]. These alterations are all supported by our current findings that TH activity is significantly inhibited at 12 weeks of age in the R6/2 mice. The decrease in TH activity likely results from the 57% loss of TH protein in the nigrostriatal terminals we observed in the R6/2 mice. A similar loss of striatal TH immunoreactivity has recently been observed in the striatum of R6/2 mice [1]. Although we were unable to detect enzymatically active TH in the substantia nigra of the human samples with current techniques, the decreased TH protein we observed suggests that TH activity may also be diminished in late-stage human cases of HD.

While excitotoxic mechanisms could account for reduction in TH activity seen in the R6/2 mice, we also observed significant reduction in TH mRNA in the substantia nigra of human HD brain. Htt is expressed in human substantia nigra [23]. We therefore performed reporter construct assays to determine if the expression of mutant Htt specifically interferes with TH promoter-mediated transcription, as well as

dopamine β-hydroxylase-mediated transcription. In both the HD25Q and HD103Q cell lines, induction of Htt caused a repression of both TH and DβH transcription. However, only in the HD103Q cells do we observe a significant reduction in TH-mediated transcription. Interestingly, reductions in the levels of the transcription factors, c-fos and CREB-binding protein (CBP), have been observed in HD models [30,39]. Both factors have been shown to stimulate TH gene transcription [16,26,43]. In addition, Sp1 has been shown to bind to mutant Htt [13,25]. Sp1-mediated transcription is inhibited upon the expression of mutant Htt. The TH promoter contains a GC-rich box, indicative of an Sp1 binding site, which is required for the proper transcriptional activation of the TH gene [32]. A reduction or sequestration of these key transcription factors may explain why TH mRNA is significantly reduced in the human HD substantia nigra.

We failed to see inhibition of TH activity or decrease in TH protein following the 24-h induction in either the HD25Q or HD103Q cells. The inducible Htt PC12 cell lines are tumor-derived, TH-overexpressing cell lines. It is possible that the 24-h expression of exon 1 of Htt we performed was not strong enough for us to produce changes in endogenous TH expression and activity. We were unable to assess the effect of longer exposures, as exposure of PC12 cells to huntingtin for more than 24 h was toxic. We believe that the decreases in TH activity and protein we observe in the 12-week-old R6/2 mice are due to a more prolonged mutant Htt expression.

One of the mysteries of HD is how a mutation in a single protein can produce so many perturbations in the HD brain. Transcriptional dysregulation is increasingly recognized to be an important pathological mechanism in HD pathogenesis [7]. Mutant huntingtin alters the expression of numerous mRNAs in a number of experimental systems, including mRNAs encoding for neurotransmitters, neurotransmitter synthetic enzymes, and neurotransmitter receptors [9,27]. Tyrosine hydroxylase thus joins an expanding list of critical molecules whose transcription is altered by mutant huntingtin.

In summary, our results indicate that expression of Htt in cellular, animal and human HD models has profound, yet heretofore undetermined effects on the dopaminergic system. The neuropathological damage seen in the substantia nigra is important because it suggests that HD cellular atrophy/pathology extends well beyond the striatum and cortex. It remains to be seen if TH activity is significantly altered in human HD patients or other polyglutamine diseases; however, pharmacological intervention to restore/maintain TH activity in the symptomatic R6/2 mice may prove beneficial in delaying the onset of HD symptoms in these mice.

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