Research Paper
Article de recherche

Protein kinase C βII mRNA levels decrease in the striatum and cortex of transgenic Huntington's disease mice

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Huntington's disease (HD) is caused by the inheritance of the *huntingtin* gene with an expanded CAG repeat. The function of the normal or mutant form of the huntingtin protein remains to be determined. We used differential display to determine differences in steady-state mRNA levels between wild-type and the R6/2 transgenic mouse model of HD. Using this method, we determined that the steady-state mRNA levels of protein kinase C βII (PKCβII) subunit are decreased in symptomatic HD mice compared with age-matched wild-type controls. The decrease in PKCβII mRNA levels occurred in both the striatum and cortex. Previously, it had been demonstrated that PKCβII immunoreactivity is decreased in the caudate-putamen of patients with Huntington's disease. PKC has been implicated in the long-term potentiation model of brain plasticity and learning, and the loss of PKC may affect information storage in HD. The expression of *htt-HD* throughout the brain affects the transcription of specific genes in regions not associated with widespread neurodegeneration.

La chorée de Huntington (CH) est causée par le gène de la protéine *huntingtine* dont le sujet a hérité avec une répétition anormale du triplet CAG. La fonction de la forme normale ou mutante de la protéine huntingtine reste à déterminer. Nous avons utilisé l'affichage différentiel pour déterminer les différences des niveaux stables d'ARNm entre le modèle murin transgénique R6/2 et le modèle murin de type sauvage de la CH. Nous avons utilisé cette méthode pour déterminer que les niveaux stables de protéines kinases C βII (PKCβII) dans l'ARNm diminuent chez les souris CH symptomatiques comparativement aux témoins de type sauvage jumelés selon l'âge. La baisse des taux de PKCβII dans l'ARNm s'est produite à la fois dans le striatum et le cortex. On avait démontré auparavant que l'immunoréactivité de la PKCβII diminue dans le putamen caudé chez les patients atteints de la chorée de Huntington. On a impliqué la PKC dans le modèle de synergie à long terme de la plasticité cérébrale et de l'apprentissage, et la perte de PKC peut avoir un effet sur le stockage de l'information chez les sujets atteints de CH. L'expression de la protéine hht-CH dans tout le cerveau affecte la transcription de certains gènes dans les régions qui ne sont pas associées à la neurodégénérescence généralisée.

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Medical subject headings: disease models, animal; genetics; Huntington disease; mice; neurodegenerative diseases; protein kinase C; RNA, messenger; transcription genetic

J Psychiatry Neurosci 2001;26(2):117-22.

Submitted May 9, 2000
Accepted Sept. 26, 2000

Introduction

Huntington's disease (HD) is caused by the inheritance of a single copy of the *huntingtin* gene (*IT-15*) with an expanded CAG repeat within exon 1 (*htt-HD*).[1,2] *htt-HD* encodes a protein with an expanded polyglutamine tract. Huntingtin, in the normal or mutant form, is widely expressed throughout the brain and body.[3–7] The expression of *htt-HD* eventually leads to progressive, but selective, neuronal loss of the γ-aminobutyric acid (GABA)- and enkephalin-containing medium spiny projection neurons of the caudate-putamen.[8] However, patients present with cognitive and psychiatric symptoms before such selective cell loss,[9,10] suggesting that neuronal dysfunction before neurodegeneration contributes to the symptoms of HD.

The function of the normal and mutant form of huntingtin remains to be defined. Based on the inheritance pattern of HD, htt-HD with the expanded polyglutamine tract has a function distinct from that encoded by the wild-type *huntingtin* gene.[2] It has been suggested that htt-HD may affect transcription by forming aberrant protein–protein interactions in the nucleus. The protein interaction domains of several transcription factors are glutamine-rich and bind other proteins through polar zippers.[11–15] The presence of extended polyglutamine tracts within proteins are not always pathogenic, however, as there are a number of proteins, including transcription factors, with extended polyglutamine tracts resident within all neurons. The development of the specific pathology and symptoms of HD, therefore, is dependent on the expression of an expanded polyglutamine tract in the huntingtin protein which exerts its effect in a cell-specific and time-dependent manner.

The first transgenic mouse model of HD was created by inserting a copy of the 5′ untranslated region, exon 1 with 145 CAG repeats and part of intron 1 of the human *huntingtin* gene, into the mouse genome.[16] The mice of one such transgenic line (R6/2) exhibit a rapidly progressing neurological phenotype beginning at about 8 weeks of age.[16,17] This phenotype includes a movement disorder characterized by shuddering, resting tremor, epileptic seizures and stereotyped behaviour. Brains of R6/2 transgenic HD mice are reduced in size compared with age-matched wild-type mice, but there is no neuronal cell loss, reactive gliosis or inflammation.[16] Cannabinoid receptor loss is one of the first molecular changes documented in HD.[8,18] The R6/2 transgenic mice have a reduction in the steady-state levels of the brain-specific cannabinoid receptor mRNA. R6/2 mice are, therefore, an experimental model of the early stages of HD before neuronal degeneration. Based on the hypothesis that mutant huntingtin may alter transcription, we used differential display to determine differences in the steady-state levels of mRNA between wild-type and R6/2 transgenic HD mice.

Materials and methods

Animals

Animal care was given according to protocols approved by Dalhousie University and the Canadian Council on Animal Care. Wild-type (B6CBAF1) and HD transgenic [B6CBA-TgN(Hdexon1)62Gpb] mice (Jackson Laboratory, Bar Harbor, Me.) were used in this study. The procedures used to determine the genotype of the mice and isolate RNA are described elsewhere.[19]

Differential display reverse transcription–polymerase chain reaction (RT–PCR)

Total cellular RNA was isolated from striatum and cortex of 10-week-old wild-type (n = 3) and 10-week-old HD transgenic (n = 3) mice using Trizol™ reagent (Gibco BRL, Burlington, Ont.) treated with RQ1 RNase-free DNase (Promega, Madison, Wis.) in the presence of RNasin™ (Promega) RNase inhibitor to remove trace genomic DNA and was then converted to single-stranded cDNA. The conditions for differential display RT–PCR are described in Denovan-Wright et al.[20]

A differential display band, amplified using primers P7 (5′-ATT AAC CCT CAC TAA ATG CTG TAT G-3′) and T8 (5′-CAT TAT GCT GAG TGA TAT CTT TTT TTT TGC-3′), was present in wild-type but greatly reduced in the single-stranded cDNA samples derived from HD mice. The PCR product was excised from the dried gel, reamplified using the P7 and T8 primers and cloned into the vector pGem-T (Promega). Plasmid DNA was isolated from selected transformants using QIAGEN spin columns. The sequence of the insert was determined using universal primers and the T7 sequencing kit (Amersham Pharmacia Biotech, Piscataway, NJ).

Northern blot

A Northern blot was prepared by size-fractionating

5 μg of total cellular RNA, isolated from the striatum and cortex of 10-week-old wild-type (n = 3) and 10-week-old HD (n = 3) mice on a 1% denaturing formaldehyde agarose gel, following standard protocols. The cloned protein kinase C βII (PKCβII) partial cDNA was used as a northern hybridization probe in conditions described previously.[21] Following hybridization of the PKCβII cDNA probe, a 372-bp cloned PCR product consisting of nucleotides 43–413 of the mouse cyclophilin cDNA (GenBank accession no. X52803) was allowed to anneal with the same Northern blot to determine the relative amounts of mRNA present in each lane. Optical densities (ODs) of the hybridization signals were determined using Molecular Analyst (Bio-Rad Laboratories, Mississauga, Ont.). ODs of cyclophilin-specific and PKCβII-specific hybridization signals were determined for each of 3 samples derived from individual animals. The local film background was subtracted from each OD value.

t-*test analysis*

The OD values for 3 samples per group for the Northern blot analysis were corrected for local background and the means subjected to Student's t-test analysis (1-tailed) with the assumption that the variances were equal. An F-test using an alpha level of 0.005, performed on the same data sets, indicated that, in all cases, the equal variance assumption was valid.

Results

PKCβII mRNA levels are reduced in the striatum and cortex of transgenic HD mice

We employed differential display RT–PCR to compare the patterns of striatal and cortical gene expression of 10-week-old wild-type and R6/2 transgenic HD mice. The majority of the PCR products generated using multiple primer combinations were common to and equal in intensity (data not shown). However, 1 PCR band generated with the P7 and T8 primers was more abundant in each of 3 samples derived from the striatum of wild-type mice than those derived from HD mice (Fig. 1).

The ~150-bp differential display band was isolated, reamplified, gel-purified and used as a hybridization probe in Northern blot analysis of total RNA isolated from the striatum and cortex of the 3 wild-type and 3 HD mice. The hybridization probe annealed with transcripts of approximately 9.5 kb in the samples derived from striatal and cortical tissue (data not shown). We cloned the differential display PCR products and de-

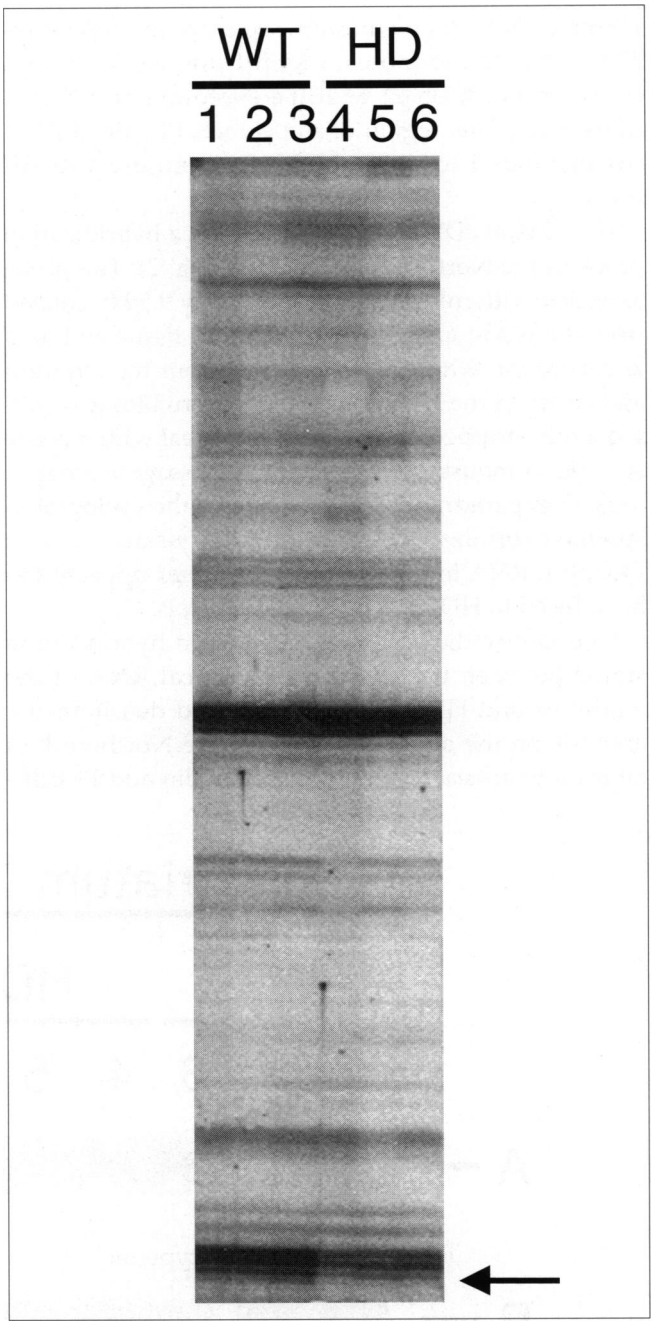

Fig. 1: A differential display band of approximately 150 bp was amplified from cDNA made from the striatal RNA of 3 10-week-old wild-type (WT; lanes 1–3) and 3 Huntington's disease (HD; lanes 4–6) mice. The relative intensity of this band (arrow) was decreased in each of the samples derived from striatal tissue of a 10-week-old HD mouse.

termined the sequence of a number of independent clones. Each of these partial cDNAs were identical in sequence. Comparison of the sequence of the cDNAs to those in GenBank revealed that the differential display band corresponded to the PKCβII mRNA. The 178-bp cDNA was identical in sequence to nucleotides 2742–2899 of the mouse PKCβII mRNA (GenBank accession no. X53532) with the exception of 2 C-to-T transitions. This region of the cloned PKCβII mRNA corresponded to the 3′ UTR of the mouse PKCβII mRNA.

The PKCβII cDNA clone was used as a hybridization probe in the Northern blot analysis (Fig. 2). The probe annealed with mRNA of approximately 9.5 kb isolated from the wild-type striatal and cortical tissue and, to a lesser extent, with mRNA isolated from the striatum and cortex of the HD mice. The Northern blot was subsequently stripped and allowed to anneal with a probe specific to mouse cyclophilin; this message is ubiquitous. Compared with the intensity of the cyclophilin-specific hybridization signal, the steady-state levels of PKCβII mRNA in the striatum and cortex appeared to be reduced in HD mice relative to controls.

To quantify the apparent decrease in hybridization signal between the striatal and cortical RNA of the wild-type and HD mice, we performed densitometric analysis on the autoradiograms of the Northern blot allowed to anneal to both the cyclophilin and PKCβII-specific probes. There was no significant difference between the OD of the bands resulting from the annealing of the cyclophilin probe. In contrast, there were significant differences in the PKCβII-specific hybridization signals between the RNA samples derived from the striatum of wild-type and HD mice ($p = 0.004$) and between the RNA samples derived from the cortex of wild-type and HD mice ($p = 0.023$). The mean PKCβII-specific OD (minus film background) of striatal RNA of the wild-type mice was 0.353 (standard deviation [SD] 0.035) and of the HD mice was 0.193 (SD 0.047) — a decrease of approximately 45%. Similarly, the mean PKCβII-specific OD (minus film background) of the wild-type cortical RNA was 0.320 (SD 0.026) and of the HD cortical RNA was 0.213 (SD 0.059), for a decrease of approximately 30%.

Discussion

We have demonstrated that the steady-state mRNA levels of PKCβII are reduced by approximately 50% and 30% in the striatum and cortex, respectively, of symptomatic HD mice compared with age-matched, wild-type mice. In addition to these findings, it has been shown previously that specific neurotransmitter receptors have diminished ligand binding or reduced steady-state mRNA levels in HD transgenic mice; Cha et al[22] surveyed the receptor-binding profiles and

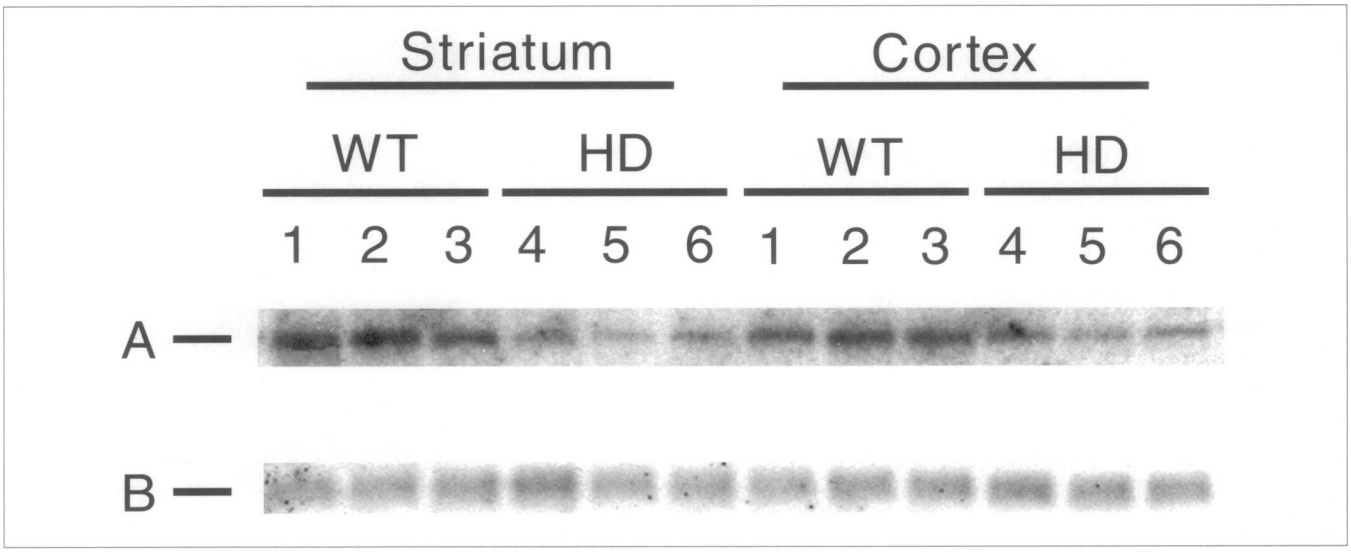

Fig. 2: Northern blot analysis using the cloned protein kinase C (PKC) βII differential display band (A). After the hybridization of the PKCβII cDNA probe, the Northern blot was allowed to anneal with a probe specific for mouse cyclophilin (B). Densitometric analysis of the PKCβII- and cyclophilin-specific hybridization signal demonstrated that the relative levels of the striatal and cortical PKCβII mRNA were reduced in symptomatic HD mice.

mRNA expression of several classes of neurotransmitters in R6/2 HD mice and found that, within the medium spiny projection neurons of the striatum, some receptor levels are normal (AMPA, NMDA-NR1, mGluR5, $GABA_A$), whereas others are decreased (mGluR1, mGluR3, DARs, mAchRs) relative to controls. These decreases in receptor-binding levels in the R6/2 mice were related to changes in receptor mRNA levels.[22] The changes in mRNA levels are not caused by a generalized decrease in transcription in a subset of striatal neurons, however, because only some of the receptor mRNAs expressed in specific striatal neurons were affected by the expression of htt-HD.

Neurotransmitter receptor changes documented in post mortem examinations of patients who had HD include decreases in glutamate (GluR), dopamine (DAR), GABA and muscarinic cholinergic (mAChR) receptors.[23–26] Decreases in cannabinoid receptor ligand binding are one of the earliest changes in human HD,[8,18] and we have demonstrated that there is a progressive decline in steady-state mRNA levels of the cannabinoid receptor (CB1) in transgenic HD mice.[19] CB1 mRNA is expressed throughout the striatum, cortex and hippocampus of wild-type mice. At 4 and 5 weeks of age, there is no difference in the distribution of the CB1 mRNA between the wild-type and transgenic HD mice. At 6, 7, 8 and 10 weeks of age, however, the HD mice exhibit reduced levels of CB1 mRNA in the lateral striatum compared with age-matched controls. The HD mice also show a loss of CB1 mRNA within a subset of neurons in the cortex and hippocampus. In other regions of the brain, including Ammon's horn of the hippocampus and the medial striatum, the levels of CB1 mRNA do not differ between wild-type and transgenic HD mice. Also, the levels of DARPP-32 mRNA decline over time in HD mice.[27] Our results demonstrate that the single-copy CB1 gene, like the single-copy DARPP-32 gene, is subjected to cell-specific and time-dependent regulation of the steady-state level of its gene product as a result of the expression of the HD gene.

Together, these observations demonstrate that the expression of htt-HD causes a reduction in the steady-state levels of a subset of mRNAs in striatal neurons and that these changes precede the outward expression of HD symptoms. Moreover, these data suggest that the dopaminergic and glutamatergic systems are selectively altered in R6/2 transgenic mice, as they are early in the time course of the HD disease progession in humans. For each of the changes in mRNA levels described thus far, it is apparent that the expression of htt-HD leads to the establishment of a new equilibrium value of select transcripts in these mice and not the complete loss of any of these messages.

Huntington's disease presents with slowing of mental processes, reduced mental flexibility and memory disorder.[28–30] PKC isoforms are involved in a broad range of neuronal functions, and recent studies have implicated PKC in learning and memory and in long-term potentiation.[31] PKC immunoreactivity increases in hippocampal principal cells of rats, mice and rabbits during learning tasks.[32] Persistent changes in phosphorylation of PKC substrates may be an important event in the process of information storage leading to the development of memory. Therefore, the decrease in PKC levels in HD may impair information storage. Similar to the decrease in PKC levels in HD mice reported here, PKCβII immunoreactivity is decreased in the caudate-putamen of HD patients.[33] Although death of neurons in the caudate-putamen is a hallmark of HD, the symptoms of cognitive dysfunction suggest that cortical areas are also affected during disease development.

This work demonstrates that the expression of htt-HD throughout the brain affects transcription of specific genes in regions not associated with widespread neurodegeneration.

Acknowledgements: We thank Kathleen Murphy for technical assistance.

This work was supported by grants from the Hereditary Disease Foundation and the Huntington's Disease Society of America to H.A.R. E.D-W. is an Eli Lilly Fellow for Schizophrenia Research.

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