Hyperdopaminergic Status in Experimental Huntington Disease

Ali Jahanshahi, MSc, Rinske Vlamings, MSc, Ahmet Hilmi Kaya, MD, Lee Wei Lim, MD, PhD, Marcus L.F. Janssen, MD, Sonny Tan, MD, Veerle Visser-Vandewalle, MD, PhD, Harry W.M. Steinbusch, PhD, and Yasin Temel, MD, PhD

Abstract

Huntington disease has been linked to increased dopaminergic neurotransmission in the striatum, and clinical studies have demonstrated that the associated chorea can be treated with dopamine antagonist or dopamine-depleting drugs. The origin of this hyperdopaminergic status is unknown. Because substantia nigra pars compacta and the ventral tegmental area are the main sources of striatal dopamine input, we hypothesized that changes in these regions relate to striatal dopaminergic alterations. Here, in a recently generated transgenic rat Huntington disease model that shows progressive striatal neurodegeneration and chorea, we found evidence of increased dopamine levels in the striatum. We also demonstrate more dopaminergic cells in the substantia nigra pars compacta and ventral tegmental area in these rats. These results suggest that increased striatal dopamine comes from these 2 main nuclei, and that it is not necessarily related to shrinkage of the striatum. The findings implicate increased dopamine input from these nuclei in the pathogenesis of chorea in Huntington disease.

Key Words: Chorea, Dopamine, Huntington disease, Striatum, Substantia nigra pars compacta, Tyrosine hydroxylase, Ventral tegmental area.

INTRODUCTION

Huntington disease (HD) is an autosomal dominant inherited progressive neurodegenerative disorder (1, 2). The mutation involves the expansion of the CAG trinucleotide repeat within exon 1 of the HD gene, which is on chromosome 4. This mutation encodes an extended polyglutamine stretch in the N-terminal domain of the huntingtin protein, the function of which is still poorly understood (3). Huntingtin accumulates in the brain and can have either toxic (4, 5) or protective effects (6). The prevalence of HD in Europe and both American continents is reported to be approximately 1 in 10,000 and affects both sexes with the same frequency (7). Huntington disease can become symptomatic at any age, but the peak incidence is at midadult life.

Huntington disease is characterized by striatal atrophy and loss of striatal projection neurons; striatal interneurons are relatively spared (8–10). The striatal projection neurons, also known as medium spiny neurons, can be divided into 2 groups based on their connectivity and neurochemistry. Degeneration takes place in both populations, but medium spiny neurons expressing the dopamine 2 receptors and enkephalin are more affected (11). In addition to profound striatal neuron death (12), there is also atrophy of the cerebral cortex and thinning of the underlying white matter (13). Neuronal loss in the hippocampus, cerebellum, and thalamus has also been reported (14, 15).

Huntington disease is characterized clinically by motor and nonmotor manifestations. The predominant motor signs are chorea and to a lesser extent hypokinesia. Nonmotor manifestations include cognitive dysfunction leading to dementia and emotional instability. The pathoanatomical basis for the chorea is not known, but a link with the dopaminergic system has been suggested. Early postmortem studies showed that striatal dopamine levels in the dorsal and ventral striatum were higher in HD patients than in controls (16–18), and clinical studies have shown that the chorea can be treated with dopamine antagonist or dopamine-depleting drugs (19).

The origin of elevated striatal dopamine levels in HD remains uncertain. Because the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) are the main sources of striatal dopamine (20, 21), we tested the hypothesis that elevated striatal dopamine levels are caused by changes in these regions. Using immunohistochemistry (IHC) with an antibody to tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of dopamine, we determined the numbers of TH-containing cells in the SNc and VTA in the transgenic rat model of HD (tgHD), the only experimental HD model with
chorea. Thus far, no other rodent model of HD has shown choreiform movements (22). This recently created model carries a truncated huntingtin cDNA fragment with 51 CAG repeats under control of the native rat Huntington promoter (23). The rats show slowly progressive clinical signs that include choreiform movements and cognitive and emotional alterations and exhibit progressive striatal cell loss, striatal atrophy, and cortical cell damage (22, 24–26).

MATERIALS AND METHODS

Animals

Three groups of male rats were studied: homozygous tgHD rats (n = 5), hemizygous tgHD rats (n = 5), and wild-type (WT) littermates (n = 4); they were approximately 11 months old. After genotyping, all rats were transferred from the Friedrich-Alexander University Animal Facilities (Erlangen-Nürnberg, Erlangen, Germany) to the Central Animal Facilities of Maastricht University (Maastricht, The Netherlands) and housed there. The rats were then adapted to the experimental environment in standard Makrolon cages on sawdust bedding in an air-conditioned room (~20°C). Food, standard laboratory chow (Hopefarms, Woerden, The Netherlands), and water were available ad libitum. All experimental procedures were approved by the Animal Experiments and Ethics Committee of Maastricht University, Maastricht, The Netherlands.

Tissue Collection

The rats were killed after deep anesthesia with Nembutal (75 mg/kg), and their brains were quickly removed and immersion fixed for 48 hours in 4% paraformaldehyde in 0.1 mol/L sodium phosphate buffer (pH 7.6). The brains were then immersed fixed overnight in 15% sucrose for cryoprotection. Selected regions of the brains were then cut serially in 30-μm-thick coronal sections on a cryostat (MICROM HM 520, Neuss, Germany) and stored at −80°C until processing for TH IHC.

Immunohistochemistry

To evaluate the number of TH-immunoreactive (IR) cells in the SNc and VTA and the level of TH expression in the dorsal and ventral striatum, we processed sections containing the SNc, VTA, and striatum for IHC. This was carried out by incubation of the sections with a mouse anti-TH antibody (diluted 1:100; kindly supplied by Dr C. Cuello, Montreal, Canada) (27). After rinsing steps with Tris-buffered solution and Triton X-100 and incubation with the secondary antibody (diluted 1:400 donkey anti-mouse biotin; Jackson ImmunoResearch Laboratories, West Grove, PA), the sections were incubated with an avidin-biotin-peroxidase complex (diluted 1:800; Elite ABC-kit, Vector Laboratories, Burlingame.

FIGURE 1. Representative low-power photomicrographs of frontal brain sections (−5.2 mm anteroposterior from bregma) stained for tyrosine hydroxylase (TH) show the substantia nigra pars compacta (SNc), optic tract (OT), and a small part of the ventral tegmental area (VTA) of a wild-type littermate rat (C), hemizygous transgenic Huntington disease (HD) rat (+/−), and a transgenic HD homozygous (+/+) rat. There is greater TH-immunoreactive (IR) cell density in the SNc of the hemizygous and homozygous transgenic HD rats than in the control. The higher power inset in the right upper corner shows a magnification of a TH-IR cell of a transgenic homozygous rat. Scale bars = 250 μm.
To visualize the horseradish peroxide reaction product, the sections were incubated with 3,3'-diaminobenzidine tetrahydrochloride with nickel chloride enhancement. Finally, the sections were mounted and coverslipped using Permount (Fisher Scientific).

**Quantitative Analysis of TH-IR Cells in the SNc and VTA**

The immunostained sections were used to evaluate the total number and volumes of TH-IR cells within the SNc and VTA. All stereological investigations were carried out with a stereological computer microscopy system (Stereo Investigator, Microbrightfield Bioscience, Williston, VT). In all sections showing the SNc and VTA, the region comprising the TH-IR cells within the SNc and VTA, respectively, was delineated, and total numbers of TH-IR cells and the cell volumes were estimated with the optical fractionator (28–30). Stereological counting method details have been previously described (27).

**Quantitative Analysis of the TH Levels in the Dorsal and Ventral Striatum**

The TH expression levels in the dorsal and ventral striatum (nucleus accumbens [NAc] and olfactory tubercle) were also evaluated. The TH expression levels were measured in the dorsal and ventral striatum (nucleus accumbens [NAc] and olfactory tubercle) of wild-type littermate rats (C), hemizygous transgenic Huntington disease (HD) rat (+/-), and a transgenic HD homozygous (+/+). There was a greater TH-immunoreactive (IR)-containing cell density in the VTA of the +/- and +/+ rats; the background fiber staining is comparable. High-power photomicrograph (inset in right upper corner) shows a magnification of TH-IR cells of a +/- rat. Volumes of TH-IR cells were not different among the groups. Scale bars = 150 μm according to (31).

---

**FIGURE 2.** (A, B) Numbers of tyrosine hydroxylase-immunoreactive (TH-IR) cells (mean ± SEM) in the substantia nigra pars compacta (SNc, A) and ventral tegmental area (VTA, B). Homozygous (+/+), hemizygous transgenic Huntington disease rats have more TH-IR neurons than wild-type littermates (C). *p < 0.05. (C, D) Cumulative volume measurements of TH-IR cells in SNc (C) and VTA (D). There are no significant differences among the groups.

**FIGURE 3.** Representative low-power photomicrographs of frontal brain sections (~5.6 mm anteroposterior from bregma) stained for tyrosine hydroxylase (TH) showing the ventral tegmental area (VTA), optic tract (OT), and mammillary tubercle (MT) of a wild-type littermate rat (C), hemizygous transgenic Huntington disease (HD) rat (+/-), and a transgenic HD homozygous (+/+) rat. There is a greater TH-immunoreactive (IR)-containing cell density in the VTA of the +/- and +/+ rats; the background fiber staining is comparable. High-power photomicrograph (inset in right upper corner) shows a magnification of TH-IR cells of a +/- rat. Volumes of TH-IR cells were not different among the groups. Scale bars = 150 μm according to (31).
were quantified using an image analysis system (analySIS Imaging System, Munster, Germany) from digital photos taken by an Olympus U-CMAD-2 digital camera connected to an Olympus AX 70 microscope (Olympus, Zoeterwoude, The Netherlands). Densitometric measurements (ImageJ software version 1.38x; National Institutes of Health, Bethesda, MD) were obtained at 2 anteroposterior levels for the dorsal and 1 level for the ventral striatum and for the right and left hemispheres. The coordinates were from bregma for the dorsal striatum +0.20 and +1.00, and for the ventral striatum +1.70 (31). Data are expressed as optical density ratios.

Statistical Analysis

Data are presented as mean ± SEM. The quantitative data of the total number of TH-IR cells, the volumes of the TH-IR cells in the SNc and VTA, and the TH expression levels in the dorsal and ventral striatum were analyzed using 1-way analysis of variance with a Bonferroni post hoc multiple comparisons test. All statistical analyses were performed with SPSS 15.0 version for Windows. p < 0.05 was considered significant.

RESULTS

TH-IR Cells in the SNc

Qualitative inspection of the stained sections showed more TH-IR cells in the SNc of tgHD rats compared with WT littermates (Fig. 1). Quantitative analysis revealed that there were no significant differences between the right and left SNc in all the groups, and therefore the data were pooled.

The mean number of TH-IR cells was 9,745 in WT littermates, 15,351 in the hemizygous tgHD rats, and 14,180 in the homozygous tgHD rats (Fig. 2). The numbers of TH-IR cells in the hemizygous and homozygous tgHD rats were significantly higher than in the WT littermates (F = 9.43, p < 0.01). There were no significant differences between hemizygous and homozygous rats.

The volumes of the TH-IR cells of the right and left SNc were not statistically different in all the groups, and therefore these data were pooled. The mean volumes of TH-IR cells were 2,939 μm³ in WT littermates, 2,681 μm³ in the hemizygous tgHD rats, and 3,072 μm³ in the homozygous tgHD rats. There were no significant differences among the groups (Fig. 2).

TH-IR Cells in the VTA

Qualitative evaluation of stained sections clearly showed more TH-IR cells in the VTA of tgHD rats versus WT littermates (Fig. 3). Quantitative analysis revealed no significant differences between the right and left VTA in all the groups, and therefore these data were pooled.
differences between right and left VTA in all groups, and the data were pooled.

The mean numbers of TH-IR cells in the VTA were 3,901 in WT littermates, 5,056 in hemizygous tgHD rats, and 5,726 in homozygous tgHD rats (Fig. 2). The number of TH-IR cells in the tgHD rats was significantly higher versus WT littermates ($F = 9.09, p < 0.01$), with no significant differences between hemizygous and homozygous animals.

The volumes of the TH-IR cells of the right and left VTA were also not statistically different in all the groups, and these data were pooled (Fig. 1). The mean volumes of TH-IR cells were 1,546 $\mu$m$^3$ in WT, 1,376 $\mu$m$^3$ in hemizygous tgHD, and 1,667 $\mu$m$^3$ in homozygous tgHD rats, with no significant differences among the groups.

TH Expression Levels in the Dorsal and Ventral Striatum

Qualitative examination of TH IHC showed clear differences between tgHD rats and WT littermates in both the dorsal and ventral striatum (Figs. 4 and 5). Quantitative analysis revealed no differences between the right and left striatum or between the 2 anteroposterior levels of the dorsal striatum, and therefore the data were pooled.

Optical density measurements showed that the TH expression in the dorsal striatum was significantly higher in tgHD rats versus WT rats ($F = 8.90, p < 0.01$); there were no differences between hemizygous and homozygous rats. Similarly, TH expression in the ventral striatum was significantly higher in tgHD rats than in their WT littermates ($F = 4.56, p < 0.05$), with no differences between hemizygous and homozygous rats (Fig. 6).
FIGURE 7. Representative high-power photomicrographs of frontal brain sections stained for tyrosine hydroxylase (TH) showing the substantia nigra pars compacta (I–III) and the ventral tegmental area (IV–VI) of a wild-type littermate rat (C), hemizygous transgenic Huntington disease (HD) rat (+/−), and a transgenic HD homozygous (+/+ ) rat. There is a greater density of TH-immunoreactive cells in the hemizygous and homozygous transgenic HD rats than in the controls. Scale bars = 75 μm.
The SNc–Dorsal Striatum (Nigrostriatal) Dopaminergic System Versus the VTA–Ventral Striatum (Mesolimbic) Dopaminergic System

Although the dopaminergic efferents from the SNc predominantly innervate the dorsomedial striatum, the medial part also innervates the ventral striatum (20). The dopaminergic efferents from the VTA innervate the rest of the ventromedial striatum. To study the changes between the groups and the anatomical subregions in more detail, we calculated and analyzed the difference scores for all the groups. This was done to determine whether there was a difference in the changes in the VTA–ventral striatum dopaminergic system when compared with the SNc–dorsal striatum dopaminergic system. The amount of increases in the number of TH-IR cells and the level of TH expression were similar for both systems.

Increase in Cell Density

Although semivolumetric analysis of the VTA and SNc revealed no differences among the groups, the densities of TH-IR neurons in these regions were greater in the transgenic rats (Fig. 7).

DISCUSSION

We demonstrate greater TH expression in the striatum of tgHD rats. Because TH enzymatic activity highly corresponds to the cellular levels of dopamine, this reflects increased striatal dopamine levels (32). This is in line with earlier postmortem HD studies that reported higher levels of dopamine in HD brains versus controls. Using a radioenzymatic detection method, Spokes (18) found greater levels of dopamine in the striatum (69% increase in the putamen, 32% in the caudate nucleus), NAc (87% increase), and the SNc (34%) in postmortem material of 56 HD patients versus controls (18). Another study with fewer subjects found no changes in dopamine levels in postmortem HD patients (33), but Bird (16) reported increased TH activity in the striatum, NAc, and substantia nigra, supporting the findings of Spokes (18). Higher levels of striatal dopamine were explained by a greater density of dopaminergic terminals in the striatum, which undergoes shrinkage; however, this could not explain the increase in the NAc and the substantia nigra, which do not show clear shrinkage in HD (16, 18). Indirect evidence for increased striatal dopamine levels is also derived from receptor studies. In tgHD rats (3) and in HD patients (34), it has been shown that dopamine 1 and dopamine 2 receptors in the striatum are significantly decreased.

Our findings suggest that the increased striatal dopamine comes from the 2 main nuclei supplying the striatum with dopamine, the SNc for the nigrostriatal dopamine pathway and the VTA for the mesolimbic dopamine pathway; therefore, the increase is not likely related to shrinkage of the striatum. Stereological counts of TH-IR cells revealed a substantial increase in numbers of TH-IR cells in both regions in tgHD rats versus controls. Because it has been demonstrated that the total number of cells in the substantia nigra in postmortem HD brains was not different from age-matched controls (35), it is possible that the increased number of TH-IR cells in the SNc and VTA is the result of a change in phenotype of the non–TH-IR cells. For the SNc, it is known that about 45% of the cells are non–TH-IR (27). A change in the phenotype of a TH cell (while the cell is still functional) has been documented in the substantia nigra of mice (36) and rats (37).

There are 2 popular hypotheses for the consequences of a hyperdopaminergic status in HD: chorea induced by increased dopamine levels and higher dopamine leading to enhanced neurodegeneration. Evidence for the first hypothesis is mainly derived from Parkinson disease and clinical observations in HD. One of the major clinical signs in Parkinson disease is hypokinesia, which has been linked to dopaminergic neuron loss in the SNc and can be treated successfully with dopamine agonists and l-dopa. In line with this, chorea in HD (which is a hyperkinesia) can be treated with dopamine antagonists and dopamine-depleting drugs. However, HD patients can also experience a certain extent of bradykinesia and rigidity. The second hypothesis is mainly supported by experimental studies; it states that higher concentrations of dopamine in the striatum lead to neurotoxicity and that this plays a role in HD pathogenesis. Dopamine can auto-oxidize to form dopamine quinone, a reactive molecule that spontaneously decomposes to form additional reactive species that can induce cell damage (38, 39).

An interesting but provocative opinion is that increased dopamine plays a role in chorea and not so much in neurodegeneration. Findings from transgenic mouse models of HD support this. For example, in R6/2 (40, 41) and R6/1 (42) HD model mice, lower dopamine levels were found in the striatum, whereas these mice show neurodegeneration but no chorea. In line with this, the tghD rats (as demonstrated in the present study) show neurodegeneration but also chorea and have a hyperdopaminergic status.

We did not find a difference in the number of TH-IR cells between homozygous and hemizygous animals, although they may exhibit differences in behavioral performance at the age of 11 months (25). Interestingly, at this age, there is also no difference in the number of choreiform movements, which arises at later ages (22, 43). This again supports the opinion that dopamine contributes to the chorea.

In conclusion, we find evidence for increased dopamine levels in the striatum in a recently generated transgenic rat model of HD that shows progressive neurodegeneration and chorea, which is consistent with postmortem human studies. Furthermore, our results show that the origin of this hyperdopaminergic status lies in increased numbers of dopaminergic cells in the VTA and SNc, the 2 main nuclei that provide the striatum with dopamine.

REFERENCES


© 2010 American Association of Neuropathologists, Inc.