Expression of Mutant Huntingtin Blocks Exocytosis in PC12 Cells by Depletion of Complexin II*

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded CAG repeat in the *HD* gene. We reported recently that complexin II, a protein involved in neurotransmitter release, is depleted from both the brains of mice carrying the HD mutation and from the striatum of post mortem HD brains. Here we show that this loss of complexin II is recapitulated in PC12 cells expressing the HD mutation and is accompanied by a dramatic decline in Ca^{2+}-triggered exocytosis of neurotransmitter. Overexpression of complexin II (but not complexin I) rescued exocytosis, demonstrating that the decline in neurotransmitter release is a direct consequence of complexin II depletion. Complexin II depletion in the brain may account for some of the abnormalities in neurotransmission associated with HD.

Huntington's disease (HD)[1] is an autosomal dominant neurodegenerative disorder characterized by motor, emotional, and cognitive dysfunction. It is caused by an expanded CAG repeat in the *HD* gene, which encodes the widely expressed 348-kDa protein, huntingtin (htt; Refs. 1 and 2). The expanded polyglutamine repeat is likely to cause a gain of function in HD (3–5), although the mechanism underlying the pathology is unknown. The role of htt in the cell is also not clear. Htt is a cytosolic protein that is found loosely attached to synaptic vesicles (6). It is known to interact with a number of different proteins, including HAP1 (7) and HIP1 (8, 9), which themselves associate with vesicles. It has therefore been proposed that abnormal protein interactions with htt may influence neurotransmitter release or membrane recapture by endocytosis and thereby cause neuronal dysfunction (6, 10).

The R6/2 transgenic mouse expresses the first exon of the *HD* gene with an expanded CAG repeat (11). Although little

neurodegeneration is seen in this mouse prior to its premature death (usually at 14–16 weeks), its phenotype has a number of similarities with HD, for example the dominant inheritance and the progressive nature of the neurological deficits (12, 13). The mice develop normally and do not show frank motor symptoms until about 8 weeks of age (11). However, motor and cognitive deficits are apparent from about 4 weeks (12, 13), and presymptomatic alterations in long term potentiation are also seen (14). The pronounced neurological phenotype of the R6/2 mouse in the absence of neurodegeneration suggests that neuronal loss in HD is secondary to neuronal dysfunction. In support of this suggestion, motor (15, 16) and cognitive deficits (17, 18) can be detected in HD patients before neurodegenerative changes are seen.

To examine the possibility that abnormalities in neurotransmission underlie early events in the development of HD, we previously looked for changes in the brains of R6/2 mice in the levels and distribution of proteins involved in neurotransmitter release (19). The proteins studied included the soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptors (SNAREs) synaptobrevin, syntaxin, and SNAP-25, which are known to form the core of a ubiquitous membrane fusion apparatus (20), and various "accessory proteins," such as the synaptic vesicle proteins synaptotagmin, synaptophysin, and rab3A, and the cytosolic proteins α-SNAP and complexins I and II. These proteins are believed to control SNARE complex assembly and disassembly and thereby modulate neurotransmitter release (20). We found that complexin II was specifically and progressively depleted from the brains of R6/2 mice (19). Furthermore, in 16-week-old mice, complexin II appeared in a subpopulation of neuronal intranuclear inclusions, which are a characteristic feature of brains from both mouse HD models (21) and human HD patients (22). Significantly, the depletion of complexin II was also seen in the striatum of HD brains (23), the region most severely affected in HD (2). The depletion was apparent at an early stage (grade 0), before neurodegeneration is seen. Decreases in the levels of synaptobrevin 2 and rab3A were also seen in the striatum, but none of the other proteins tested was significantly affected.

In our previous studies, we established a correlation between complexin II depletion and neurological dysfunction in both HD patients and a mouse model of HD. In the present study, we sought to determine whether expression of mutant htt was able to cause complexin II depletion and whether this effect in turn compromised Ca^{2+}-triggered exocytosis.

EXPERIMENTAL PROCEDURES

PC12 Cells—PC12 cells were cultured in Dulbecco's modified essential medium, containing glucose (4.5 mg/ml), $NaHCO_3$ (3.7 mg/ml), 5% horse serum, and 5% calf serum, in 10% CO_2 air at 37 °C. Where appropriate, expression of the green fluorescent protein (GFP)-tagged

[1] The abbreviations used are: HD, Huntington's disease; CFP, cyan fluorescent protein; GFP, green fluorescent protein; htt, huntingtin; PBS, phosphate-buffered saline; SNARE, soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor; SNAP, soluble *N*-ethylmaleimide-sensitive fusion attachment protein.
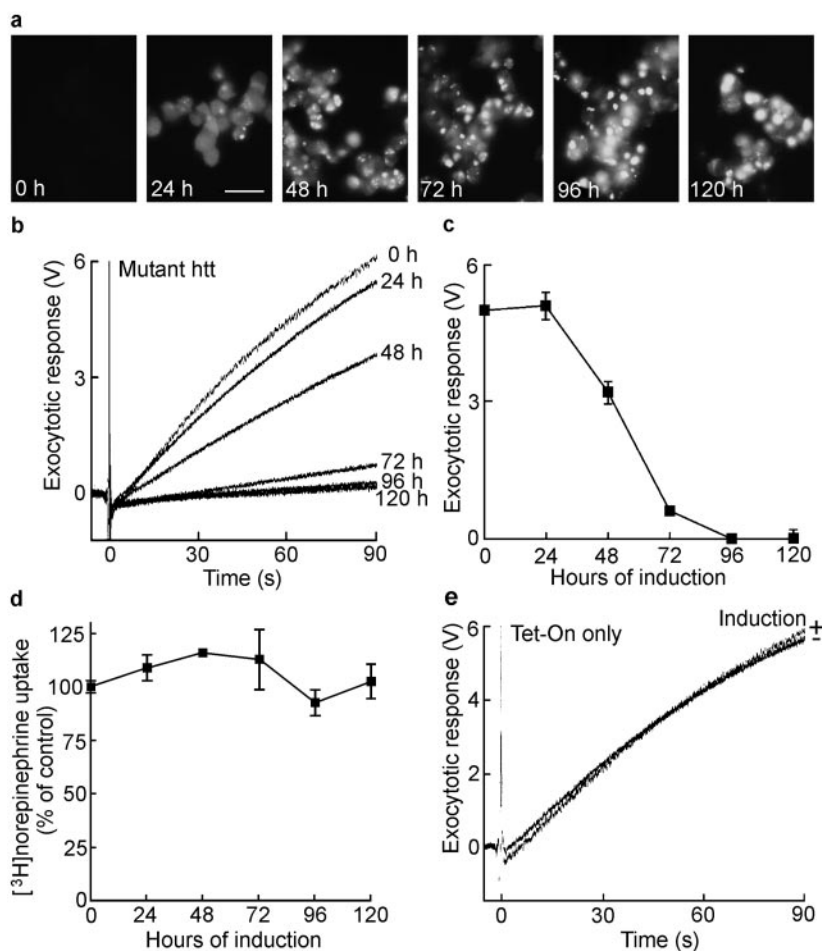
FIG. 1. **Effects of expression of mutant htt fragment on neurotransmitter release from permeabilized PC12 cells.** *a*, expression of GFP-tagged mutant htt fragment was induced by addition of doxycycline (1 μg/ml). Intranuclear inclusions were clearly visible in the majority of cells after 24 h, and the presence of inclusions was maximal after 48 h. *Scale bar*, 20 μm. *b*, real-time measurement of neurotransmitter release from cells expressing mutant htt fragment for various times. Ca^{2+} (100 μM) was added at time 0. *c*, mean values for neurotransmitter release ($\pm$S.E., $n = 4$). *d*, [^{3}H]norepinephrine uptake into cells expressing mutant htt fragment for various times, expressed as a percentage of uptake into control cells ($\pm$S.E., $n = 4$). *e*, neurotransmitter release in untreated and doxycycline-treated (120 h) cells expressing Tet-On vector alone.

mutant htt fragment via the Tet-On system (Clontech) was induced by treatment with doxycycline (1 μg/ml). For complexin overexpression experiments, the enhanced GFP coding sequence in the vector pIRES2-EGFP (Clontech) was replaced by the enhanced cyan fluorescent protein (CFP) sequence. cDNAs encoding complexins I and II (24) were then subcloned into the vector using *Eco*RI and *Bam*HI. Both constructs were validated by sequencing before use. Cells were transfected with 50 μg of DNA using a Bio-Rad electroporator set at 250 microfarads and 250 V. Transfected cells were identified through the presence of uniform cyan fluorescence.

Real-time Voltammetric Measurement of Neurotransmitter Release from Permeabilized PC12 Cells—Cells were detached and permeabilized by centrifugation at 1600 $\times$ *g* for 3 min; this procedure results in the permeabilization of ~80% of the cells, as determined by trypan blue staining (25). Cells were used immediately after permeabilization to avoid rundown in exocytosis. A cell suspension (350 μl containing ~10^7 cells), in 100 mM NaCl, 50 mM Hepes (pH 7.4), containing 1 mg/ml bovine serum albumin and 2 mM EGTA, was added to a temperature-controlled incubation chamber (containing Ag/AgCl reference and platinum auxiliary electrodes) set at 37 °C. A glassy carbon rotating disk electrode (E_{app} = +500 mV *versus* the reference electrode) was rotated in the cell suspension at 3000 rpm. Once a stable base line was obtained (usually 1 min), a CaCl$_2$ solution was rapidly injected into the suspension to achieve a free Ca^{2+} concentration of 100 μM. Catecholamine (predominantly dopamine) released from the cells is oxidized at the surface of the rotating disk electrode, and the current generated is directly proportional to the concentration of the species being oxidized.

Measurement of [^{3}H]Norepinephrine Uptake into PC12 Cells—PC12 cells were grown on polylysine-coated 24-well plates, and expression of the mutant htt fragment was induced for various times by treatment with doxycycline (1 μg/ml). Over the last 16 h of the induction period, the cells were incubated in medium containing [^{3}H]norepinephrine (Amersham Biosciences; 52 Ci/mmol, 0.4 μCi/ml), sodium ascorbate (0.5 mM), and doxycycline where appropriate. At the end of the incubation, the labeling medium was aspirated, and the cells were washed once with ice-cold 100 mM NaCl, 50 mM Hepes buffer (pH 7.4). Cells were solubilized in 0.2 ml of 0.5% Triton X-100, and the wells were washed

with a further 0.2 ml of the same solution. The cell lysates were transferred to scintillation vials and analyzed by liquid scintillation counting. Blank wells were treated identically, and the associated radioactivity was subtracted from the experimental values to give values for cell uptake of [^{3}H]norepinephrine.

Immunofluorescence Microscopy—Cells were grown on polylysine-coated glass coverslips in either the presence or absence of doxycycline (1 μg/ml). After a 72-h induction, the cells were fixed with 3.7% ice-cold formalin in phosphate-buffered saline, pH 7.4 (PBS), for 30 min. After three washes with PBS at room temperature, cells were stored in "block solution" (3% deer serum, 0.2% Triton X-100, and 0.1% sodium azide in PBS) at 4 °C until use. Cells were incubated with mouse monoclonal anti-complexin II antibody (1:1000) in block solution overnight at 4 °C, washed five times at room temperature with 0.2% Triton X-100 in PBS, and then incubated in Alexa Fluor 594-conjugated goat anti-mouse antibody (1:2000; Molecular Probes) for 1 h at room temperature. Cells were washed five times and then incubated in Hoechst 33258 dye (5 μg/ml in PBS) for 30 min at 37 °C. After another five washes, coverslips were mounted on glass slides with Pro-Long mounting reagent and examined using a Nikon Eclipse TE2000 microscope equipped with a DXM1200 digital camera.

Amperometric Detection of Neurotransmitter Release from Single Intact PC12 Cells—Catecholamine was detected as an oxidation current using 5-μm carbon fibers polarized to 650 mV. Current was recorded at room temperature (~22 °C) with a VA-10 amplifier (ALA Scientific Instruments, Westbury, NY). Signals were filtered at 1 kHz and digitized at 4 kHz for computer acquisition with PCLAMP 8 software (Axon Instruments, Foster City, CA). Cells were bathed in 150 mM NaCl, 4.2 mM KCl, 1 mM NaH$_2$PO$_4$, 0.7 mM MgCl$_2$, 2 mM CaCl$_2$, 10 mM Hepes (pH 7.4). Cells (2 $\times$ 10^5) were plated onto 35-mm dishes coated with collagen I/poly-D-lysine and loaded by bathing overnight in 1.2 mM norepinephrine and 0.4 mM ascorbate 1 day prior to recording. Release was elicited by pressure ejection of 105 mM KCl, 5 mM NaCl from a ~2-μm tipped micropipette positioned within 10 μm of the cell. Cells were transfected by electroporation using an ECM 830 electroporator (BTX, San Diego, CA) set at 230 V and 5 ms.

RESULTS

For our experiments we used a PC12 cell line stably expressing the first exon of htt with a 74-glutamine repeat and an N-terminal GFP tag, via the Tet-On system (26). Cells were treated for various times with doxycycline (1 μg/ml) to initiate expression of the protein. By 24 h, the cells showed a marked increase in GFP expression, which was accompanied by the appearance of GFP-containing aggregates ("inclusions") in some cells. By 48 h, 62% of the cells had inclusions, and this percentage remained stable thereafter (Fig. 1a). Expression of the htt fragment did not cause significant cell death, up to 120 h (data not shown). To measure neurotransmitter release, cells were permeabilized and exocytosis was triggered by addition of Ca^{2+} (100 μM). Release of catecholamine neurotransmitter was detected in real time using rotating carbon disc electrode voltammetry (25). The amount of neurotransmitter released fell progressively after expression of the HD mutation, and was almost undetectable after 72 h (Fig. 1, *b* and *c*). Over the same time course, there was no consistent change in the ability of the PC12 cells to take up [³H]norepinephrine (Fig. 1d), indicating that the fall in neurotransmitter release represented a defect in exocytosis rather than a reduction in the neurotransmitter content of the dense-core vesicles. In contrast to the result with the cells expressing the mutant htt fragment, exocytosis was not compromised in doxycycline-treated cells stably expressing the Tet-On vector alone (Fig. 1e).

Cells used in the neurotransmitter release assays were analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting with antibodies to proteins known to be involved in exocytotic membrane fusion (20). There was no time-dependent change in the overall pattern of protein expression, as revealed by Coomassie Blue staining (Fig. 2a). There was also no difference between untreated cells and cells that had expressed the mutant htt fragment for 120 h in the levels of the vesicle membrane proteins synaptobrevin 2, synaptotagmin I, synaptotagmin IX, and synaptophysin or in the plasma membrane proteins syntaxin 1 and SNAP-25 (Fig. 2b). In contrast, the cytosolic protein complexin II was dramatically depleted after 120 h. Over the time course studied, complexin II levels initially rose (at 24 h), and then fell progressively from 48 h onwards, reaching a minimum at 96 h (Fig. 2c). In the same cell samples, levels of synaptotagmin I and syntaxin 1 did not change. Furthermore, consistent with its lack of effect on exocytosis, doxycycline treatment caused no change in complexin II levels in PC12 cells expressing Tet-On vector alone (Fig. 2d).

In addition to changing complexin II levels, expression of the mutant htt fragment caused a redistribution of the protein within the PC12 cells. In untreated cells (Fig. 3, *a–c*), complexin II had a punctate distribution throughout the cytoplasm, whereas in cells treated with doxycycline for 72 h (Fig. 3, *d–f*), complexin II was predominantly present in large aggregates which partially corresponded with inclusions containing GFP-tagged mutant htt fragment, indicated by the *arrows* in Fig. 3, *d* and *e*.

To determine whether there was a causal connection between complexin II depletion and inhibition of exocytosis, we tested the ability of complexin II to "rescue" exocytosis in PC12 cells expressing the mutant htt fragment. Initially, we repeated the experiments shown in Fig. 1 and preincubated the permeabilized PC12 cells for 1 min with recombinant His_6-tagged complexin II before addition of Ca^{2+} (100 μM). No increase in neurotransmitter release was seen, irrespective of the duration of expression of the mutant htt fragment (data not shown). Hence, complexin II was not able to rescue exocytosis when provided acutely. We therefore decided to overexpress complexin II over the duration of expression of the mutant htt fragment. Cells were transiently transfected with a bicistronic
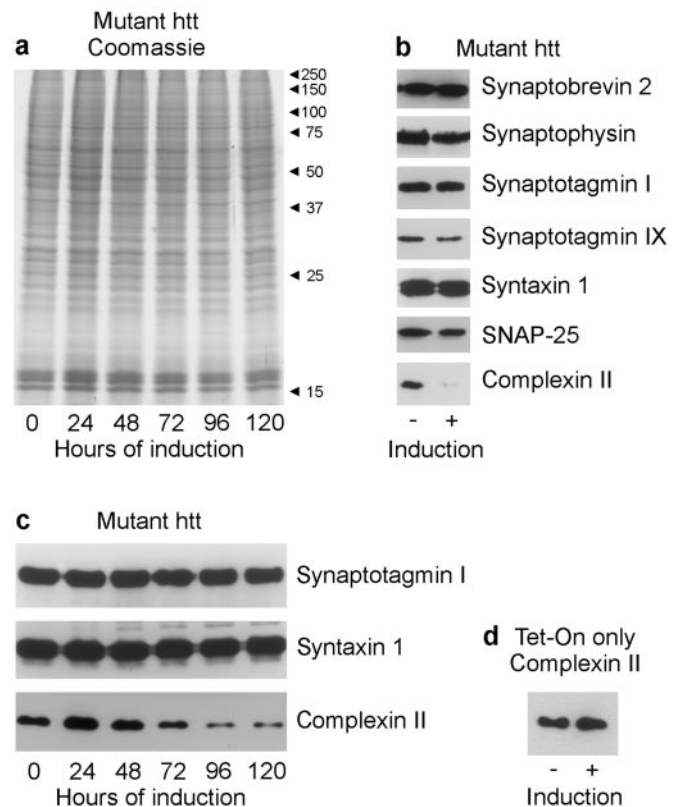

FIG. 2. **Effects of expression of mutant htt fragment on protein levels in PC12 cells.** *a*, total protein expression pattern of cells expressing mutant htt fragment for various times. Proteins were analyzed by SDS-polyacrylamide gel electrophoresis and visualized by Coomassie Blue staining. *b*, immunoblot analysis of protein expression in untreated cells and cells expressing mutant htt fragment for 120 h. *c*, immunoblot analysis of levels of synaptotagmin I, syntaxin 1 and complexin II after various times of expression of mutant htt fragment. *d*, immunoblot analysis of complexin II levels in untreated and doxycycline-treated (120 h) cells expressing Tet-On vector alone.

vector encoding both complexin II and CFP; cyan fluorescence was used to identify transfected cells. Expression of the mutant htt fragment was initiated 24 h after transfection with complexin II/CFP and doxycycline treatment continued for 72 h. Neurotransmitter release from single cells in response to KCl depolarization was measured by carbon fiber amperometry, which is able to detect the release of neurotransmitter from individual dense-core vesicles (27, 28). In cells expressing the mutant htt fragment, the amperometric recordings showed that vesicular release occurred infrequently (Fig. 4a and d), consistent with our voltammetry results showing reduced release in these cells (Fig. 1). Overexpression of complexin II caused a significant (~3-fold) increase in the number of release events evoked by depolarization (Fig. 4, *c* and *d*) and also increased the fraction of secreting cells from 50 to 85%. In contrast, overexpression of complexin I had the opposite effect (Fig. 4, *b* and *d*), reducing the cumulative spike number to about a third of that seen in control cells. However, the fraction of cells showing no secretion remained the same as controls (50%). In wild type PC12 cells, overexpression of complexin II reduced the number of evoked release events by about 50% (Fig. 4e), whereas complexin I overexpression had no effect on spike number.

DISCUSSION

The mechanism underlying polyglutamine-dependent pathogenesis in HD is unknown. Wild type htt exists predominantly in the cytoplasm (29). Mutant htt is also found in the cytoplasm; however, the N-terminal region of mutant htt, and
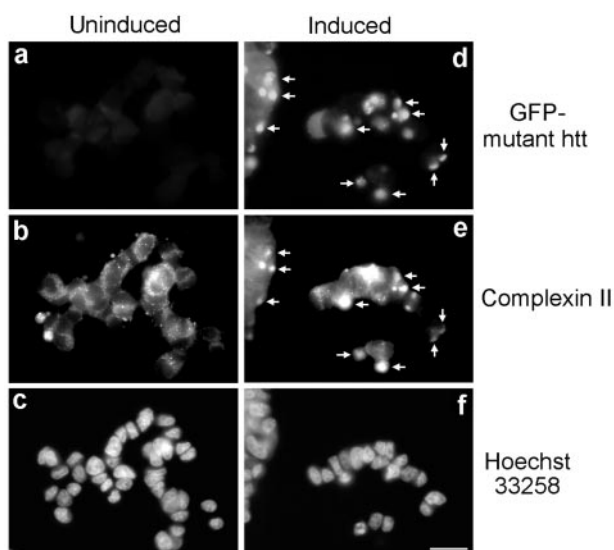
FIG. 3. **Effects of expression of mutant htt fragment on the subcellular distribution of complexin II.** The localization of complexin II was determined in untreated PC12 cells (*a–c*) and in cells in which expression of GFP-tagged mutant htt fragment had been induced for 72 h (*d–f*), using immunofluorescence microscopy. Mutant htt fragment was visualized through its GFP fluorescence (*a* and *d*). Complexin II was visualized using a mouse monoclonal anti-complexin II antibody and an Alexa Fluor 594-conjugated goat anti-mouse secondary antibody (*b* and *e*). Nuclei were revealed by staining with Hoechst 33258 (*c* and *f*). *Arrows* indicate sites of co-localization of GFP-tagged mutant htt fragment and complexin II (*d* and *e*). *Scale bar*, 20 μm.

FIG. 4. **Rescue of neurotransmitter release by complexin II.** Amperometric traces report single vesicle release events as spikes. The *bars* indicate the time of depolarization by microapplication of a KCl solution. *a*, control trace from a PC12 cell expressing the mutant htt fragment for 72 h. *b*, overexpression of complexin I (*CPX I*) failed to restore secretion. *c*, overexpression of complexin II (*CPX II*) produced a dramatic enhancement of release. *d*, cumulative spike numbers for cells expressing the mutant htt fragment are plotted *versus* time, starting with the time of KCl application (indicated by the *bar*). *e*, cumulative spike numbers for wild type cells. Where appropriate, transfections were performed 1 day prior to doxycycline treatment. All recordings were made 72 h after doxycycline addition. Three transfections were performed for each data set, with a total of 25, 20, and 33 cells in *d* and 21, 21, and 25 cells in *e*, for control, CPX I, and CPX II, respectively.

perhaps even the full-length protein, appear to be targeted to the nucleus, at least under some circumstances (30). Interestingly, mutant htt is also recruited into abnormal aggregates of protein in nuclei of neurons, both in HD brains and in mouse models of HD. The significance of these neuronal intranuclear inclusions is disputed, and evidence has been presented supporting claims that they are toxic (21), protective (31), or merely epiphenomena (32). Nevertheless, these aggregates have been isolated and shown to contain many other proteins in addition to htt (33). In light of these findings, it has been suggested that the recruitment of proteins into the inclusions, as a consequence of abnormal interactions with htt, might impact on cell function. Recent evidence has also suggested that mutant htt dysregulates transcription in neurons (34), and the expression of many genes is known to be altered in mouse models of HD (35).

In the present study, we found that the total amount of complexin II in PC12 cells expressing mutant htt fragment initially rose (at 24 h) and then fell below normal levels (from 48 h onwards). The initial rise in complexin II levels was not accompanied by any change in neurotransmitter release; however, the later complexin II depletion was associated with a concomitant fall in neurotransmitter release. In addition to the changes in total amounts of complexin II within the cells, there was an intracellular redistribution of this protein. In particular, at later times (72 h) complexin II appeared to accumulate, at least partially, in the inclusions. Similar changes (both complexin II depletion and recruitment into inclusions) have previously been seen over a much longer time course in the brains of R6/2 mice (19). Complexin II is also depleted in human HD striatum (23). Interestingly, in hippocampus from HD brains, complexin II levels were actually found to be elevated. This effect could represent a physiological rescue of synaptic transmission, since hippocampal function is not affected until late in the course of the disease.

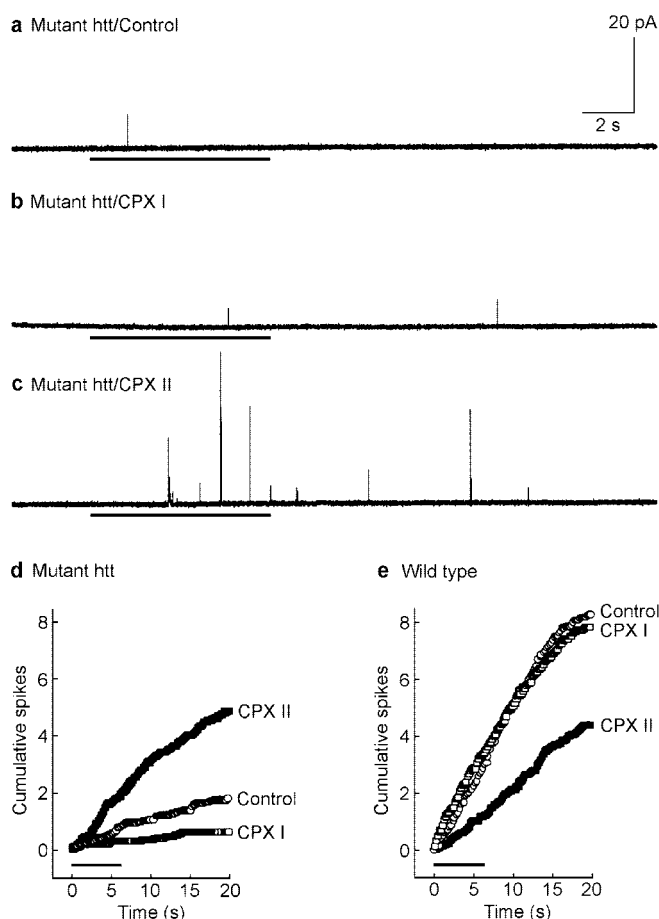The mechanism underlying the changes in complexin II levels

seen in the present study is unclear. There was no difference between complexin II mRNA levels in untreated cells and in cells treated with doxycycline for 120 h (data not shown), indicating that effects at the level of transcription are unlikely and that the depletion of the protein at later times reflects an increased rate of degradation. It is possible that the gradual recruitment of complexin II into inclusions, together with the fall in total amount of cellular complexin II, would have resulted in a depletion of this protein from the cytosol and a consequent reduction in its availability at sites of exocytotic membrane fusion. The fact that exocytosis was substantially rescued by co-expression of complexin II supports the idea that exocytosis was compromised by a lack of functional complexin II.

Complexin II binds rapidly and with high affinity to the SNARE complex (36, 37); however, its precise role in membrane fusion is still undetermined. It was originally proposed to be a negative regulator of exocytosis, based on the ability of an anti-complexin II antibody to stimulate neurotransmitter release from neurons in the *Aplysia* buccal ganglion, and of recombinant complexin II to inhibit release (38). In support of these findings, it was shown recently that overexpression of complexin II inhib-

ited exocytosis of small synaptic-like vesicles in PC12 cells (39) and of large dense-core vesicles in a related system, the adrenal chromaffin cell (40). In contrast, the deficiencies in long term potentiation in complexin II-deficient mice (41), and the reduced Ca^{2+} sensitivity of neurotransmitter release in mice lacking both complexins I and II (42), suggest that the complexins have a positive role in exocytosis. Furthermore, complexin II has recently been shown to enhance an interaction between the complementary transmembrane regions of syntaxin and synaptobrevin, suggesting a mechanism by which it could promote membrane fusion (43). In our experiments, complexin II reduced exocytosis in control PC12 cells but enhanced it in cells expressing the mutant htt fragment. Interestingly, spike numbers in response to overexpression of complexin II were similar in the wild type cells and in the cells expressing the mutant htt fragment. This result suggests that although complexin II is required to support exocytosis, there is an optimal cytosolic concentration, above which exocytosis becomes inhibited, perhaps because other crucial factors are titrated out.

Complexins I and II have distinct, but overlapping distributions within the brain (38, 42, 44). Mice lacking complexins I and II have distinct phenotypes, which might reflect the presence of the two complexins in distinct populations of neurons. Complexin I-deficient mice develop a strong ataxia and suffer from sporadic seizures, whereas complexin II-deficient mice show no obvious phenotypic abnormalities (42). In fact, defects in neurotransmission, particularly long term potentiation have been identified in complexin II-deficient mice (41). Furthermore, we have shown recently that the complexin II-deficient mice, while appearing outwardly normal, in fact show progressive deficits in a number of complex behaviors. These include abnormalities in exploration, socialization, motor coordination, and learning, suggesting that complexin II is essential for the maturation of higher cognitive functions.[2] It is particularly interesting that similar deficits are found in R6/2 mice (12, 13), which also show a progressive depletion of complexin II (19).

The close sequence similarity between complexins I and II has led to the implicit assumption that they are likely to play similar roles. However, in our study, there was a sharp contrast between the functional effects of overexpression of complexins I and II, suggesting that the two proteins have distinct molecular properties. Few studies have directly compared the properties of the two proteins, although it has been shown that complexin II binds to and dissociates from the SNARE complex more rapidly than complexin I (37). Whether this relatively minor biochemical difference can account for the significant difference in the function of the two complexins is unclear.

We have shown that expression of the HD mutation in PC12 cells causes a specific depletion of complexin II and that this depletion causes a reduction in the capacity of these cells to release neurotransmitter. Our results suggest a molecular mechanism that might account for the deficiencies in synaptic transmission that are known to occur in HD (14) and that might in turn underlie the neurological symptoms that characterize the disease (2).

REFERENCES

1. Huntington's Disease Collaborative Research Group (1993) *Cell* **72,** 971–983
2. Vonsattel, J.-P. G., and DiFiglia, M. J. (1998) *J. Neuropathol. Exp. Neurol.* **57,** 369–384
3. Duyao, M. P., Auerbach, A. B., Ryan, A., Persichetti, F., Barnes, G. T., McNeil, S. M., Ge, P., Vonsattel, J.-P., Gusella, J. F., Joyner, A. L., and MacDonald, M. E. (1995) *Science* **269,** 407–410
4. Nasir, J., Floresco, S. B., O'Kusky, J. R., Diewert, V. M., Richman, J. M., Zeisler, J., Borowski, A., Marth, J. D., Phillips, A. G., and Hayden, M. R. (1995) *Cell* **81,** 811–823
5. Zeitlin, S., Liu, J. P., Chapman, D. L., Papaioannou, V. E., and Efstratiadis, A. (1995) *Nat. Genet.* **11,** 155–163
6. DiFiglia, M., Sapp, E., Chase, K., Schwarz, C., Meloni, A., Young, C., Martin, E., Vonsattel, J.-P., Carraway, R., Reeves, S. A., Boyce, F. M., and Aronin, N. (1995) *Neuron* **369,** 493–497
7. Li, X.-J., Li, S.-H., Sharp, A. H., Nucifora, F. C., Schilling, G., Lanahan, A., Worley, P., Snyder, S. H., and Ross, C. A. (1995) *Nature* **378,** 398–402
8. Kalchman, M. A., Koide, H. B., McCutcheon, K., Graham, R. K., Nichol, K., Nishiyama, K., Kazemi-Esfarjani, P., Lynn, F. C., Wellington, C., Metzler, M., Goldberg, Y. P., Kanazawa, I., Gietz, R. D., and Hayden, M. R. (1997) *Nat. Genet.* **16,** 44–53
9. Wanker, E. E., Rovira, C., Scherzinger, E., Hasenbank, R., Walter, S., Tait, D., Colicelli, J., and Lehrach, H. (1997) *Hum. Mol. Genet.* **6,** 487–495
10. Velier, J., Kim, M., Schwarz, C., Kim, T. W., Sapp, E., and Chase, K. (1998) *Exp. Neurol.* **152,** 34–40
11. Mangiarini, L., Sathisivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trottier, Y., Lehrach, H., Davies, S. W., and Bates, G. P. (1996) *Cell* **87,** 493–506
12. Carter, R. J., Lione, L. A., Humby, T., Mangiarini, L., Mahal, A., Bates, G., Dunnett, S. B., and Morton, A. J. (1999) *J. Neurosci.* **19,** 3248–3257
13. Lione, L. A., Carter, R. J., Hunt, M. J., Bates, G. P., Morton, A. J., and Dunnett, S. B. (1999) *J. Neurosci.* **19,** 10428–10437
14. Murphy, K. P. S. J., Carter, R. J., Lione, L. A., Mangiarini, L., Mahal, A., Bates, G. P., Dunnett, S. B., and Morton, A. J. (2000) *J. Neurosci.* **20,** 5115–5123
15. Myers, R. H., Vonsattel, J., Stevens, T. J., Cupples, L. A., Richardson, E. P., Martin, J. B., and Bird, E. D. (1988) *Neurology* **38,** 341–347
16. Smith, M. A., Brandt, J., and Shadmehr, R. (2000) *Nature* **403,** 544–549
17. Foroud, T., Siemers, E., Kleindorfer, D., Bill, D. J., Hodes, M. E., Norton, J. A., Conneally, P. M., and Christian, J. C. (1995) *Ann. Neurol.* **37,** 657–664
18. Lawrence, A. D., Hodges, J. R., Rossor, A. E., Kershaw, A., ffrench-Constant, C., Rubinsztein, D. C., Robbins, T. W., and Sahakian, B. J. (1998) *Brain* **121,** 1329–1341
19. Morton, A. J., and Edwardson, J. M. (2001) *J. Neurochem.* **76,** 166–172
20. Jahn, R., and Südhof, T. C. (1999) *Annu. Rev. Biochem.* **68,** 863–911
21. Davies, S. W., Turmaine, M., Cozens, B. A., DiFiglia, M., Sharp, A. H., Ross, C. A., Scherzinger, E., Wanker, E. E., Mangiarini, L., and Bates, G. P. (1997) *Cell* **90,** 537–548
22. DiFiglia, M., Sapp, E., Chase. K. O., Davies, S. W., Bates, G. P., Vonsattel, J.-P., and Aronin, N. (1997) *Science* **277,** 1990–1993
23. Morton, A.J., Faull, R. L. M., and Edwardson, J. M. (2001) *Brain Res. Bull.* **56,** 111–117
24. McMahon, H. T., Missler, M., Li, C., and Südhof, T. C. (1995) *Cell* **83,** 111–119
25. Earles, C. A., Bai, J., Wang, P., and Chapman, E. R. (2001) *J. Cell Biol.* **154,** 1117–1123
26. Wyttenbach, A., Swartz, J., Kita, H., Thykjaer, T., Carmichael, J., Bradley, J., Brown, R., Maxwell, M., Schapira, A., Orntoft, T. F., Kato, K., and Rubinsztein, D. C. (2001) *Hum. Mol. Genet.* **10,** 1829–1845
27. Wightman, R. M., Jankowski, J. A., Kennedy, R. T., Kawagoe, K. T., Schroeder, T. J., Leszczyszyn, D. J., Near, J. A., Diliberto, E. J., Jr., and Viveros, O. H. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 10754–10758
28. Wang, C.-T., Grishanin, R., Earles, C. A., Chang, P. Y., Martin, T. F. J., Chapman, E. R., and Jackson, M. B. (2001) *Science* **294,** 1111–1115
29. Wilkinson, F. L., Man, N. T., Manilal, S. B., Thomas, P., Neal, J. W., Harper, P. S., Jones, A. L., and Morris, G. E. (1999) *Mol. Brain Res.* **69,** 10–20
30. Wheeler, V. C., White, J. K., Gutekunst, C. A., Vrbanac, V., Weaver, M., Li, X. J., Li, S. H., Yi, H., Vonsattel, J.-P., Gusella, J. F., Hersch, S., Auerbach, W., Joyner, A. L., and MacDonald, M. E. (2000) *Hum. Mol. Genet.* **9,** 503–513
31. Cummings, C. J., Reinstein, E., Sun, Y., Antalffy, B., Jiang, Y., Ciechanover, A., Orr, H. T., Beaudet, A. L., and Zoghbi, H. Y. (1999) *Neuron* **24,** 879–892
32. Saudou, F., Finkbeiner, S., Devys, D., and Greenberg, M. E. (1998) *Cell* **95,** 55–66
33. Suhr, S. T., Senit, M.-C., Whitelegge, J. P., Faull, K. F., Cuizon, D. B., and Gage, F. H. (2001) *J. Cell Biol.* **153,** 283–294
34. Dunah, A. W., Jeong, H., Griffin, A., Kim, Y.-M., Standaert, D. G., Hersch, S. M., Mouradian, M. M., Young, A. B., Tanese, N., and Krainc, D. (2002) *Science* **296,** 2238–2243
35. Luthi-Carter, R., Strand, A. D., Hanson, S. A., Kooperberg, C., Schilling, G., La Spada, A. R., Merry, D. E., Young, A. B., Ross, C. A., Borchelt, D. R., and Olson, J. M. (2002) *Hum. Mol. Genet.* **11,** 1927–1937
36. Pabst, S., Hazzard, J. W., Antonin, W., Südhof, T. C., Jahn, R., Rizo, J., and Fasshauer, D. (2000) *J. Biol. Chem.* **275,** 19808–19818
37. Pabst, S., Margittai, M., Vainius, D., Langen, R., Jahn, R., and Fasshauer, D. (2002) *J. Biol. Chem.* **277,** 7838–7848
38. Ono, S., Baux, G., Sekiguchi, M., Fossier, P., Morel, N. F., Nihonmatsu, I., Hirata, K., Awaji, T., Takahashi, S., and Takahashi, M. (1998) *Eur. J. Neurosci.* **10,** 2143–2152
39. Itakura, M., Misawa, H., Sekiguchi, M., Takahashi, S., and Takahashi, M. (1999) *Biochem. Biophys. Res. Commun.* **265,** 691–696
40. Archer, D. A., Graham, M. E., and Burgoyne, R. D. (2002) *J. Biol. Chem.* **277,** 18249–18252
41. Takahashi, S., Ujihara, H., Huang, G.-Z., Yagyu, K., Sanbo, M., Kaba, H., and Yagi, T. (1999) *Eur. J. Neurosci.* **11,** 2359–2366
42. Reim, K., Mansour, M., Varoqueaux, F., McMahon, H. T., Südhof, T. C., Brose, N., and Rosenmund, C. (2001) *Cell* **104,** 71–81
43. Hu, K., Carroll, J., Rickman, C., and Davletov, B. A. (2002) *J. Biol. Chem.* **277,** 41652–41656
44. Yamada, M., Saisu, H., Ishizuka, T., Takahashi, H., and Abe, T. (1999) *Neuroscience* **93,** 7–18

[2] A. J. Morton, unpublished results.

Expression of Mutant Huntingtin Blocks Exocytosis in PC12 Cells by Depletion of Complexin II

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