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3. ANPII was synthesized by the solid-phase method of Merrifield (*10*). The detailed synthesis of APII has been described (*11*). Synthetic APII was purified by high-pressure liquid chromatography (HPLC) and Sephadex chromatography and was shown to be a single peptide identical to natural APII by thin-layer chromatography (three different solvent systems) and HPLC (four different systems, two solvents and two columns). The homogeneity of the compound was also confirmed by comparison with HPLC of reduced APII, APII after oxidation, and reduced APII obtained by treating APII with dithiothreitol. The structure of synthetic APII was confirmed by amino acid analysis (both before and after oxidation of sulfhydryl groups to disulfide), fast atom–bombardment mass spectrometry, gel filtration, and also, independently, by amino acid sequencing. In addition, the native and synthetic APII had identical effects in rabbit thoracic-aortic strips contracted with serotonin, phenylephrine, prostaglandin F_2, and Ca^{2+} and in pulmonary arteries (*12*, *13*). The cystine disulfide bridge of APII was reduced with one equivalent of dithiothreitol. The reduced APII was purified by HPLC.
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6. The dogs were anesthetized with pentobarbital sodium (30 mg/kg intravenous injection), and cannulae were placed in the femoral arteries, a femoral vein, the right atrium, and a branch of the left coronary artery. The coronary artery was perfused at constant flow rate with heparinized blood diverted from a femoral artery by a Masterflex roller pump. Coronary perfusion was adjusted until pressures were equal in the femoral and coronary arteries in the control state. The observed variable was the change in coronary perfusion pressure during bolus injection of APII into the coronary perfusion line. The weight of cardiac tissue to which APII was administered was determined by administration of crystal violet dye into the perfusion cannula and dissection and weighing of the dyed region.
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8. This estimate assumes that 5 percent of body weight is plasma, that 60 percent of blood is plasma, that ANP is confined to plasma for 1 minute, and that rat-heart blood flow is 3.3 ml/min-g (*7*).
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Selective Sparing of a Class of Striatal Neurons in Huntington's Disease

Abstract. *A distinct subpopulation of striatal aspiny neurons, containing the enzyme nicotinamide adenine dinucleotide phosphate diaphorase, is preserved in the caudate nucleus in Huntington's disease. Biochemical assays confirmed a significant increase in the activity of this enzyme in both the caudate nucleus and putamen in postmortem brain tissue from patients with this disease. The resistance of these neurons suggests that the gene defect in Huntington's disease may be modifiable by the local biochemical environment. This finding may provide insight into the nature of the genetically programmed cell death that is a characteristic of the disease.*

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Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder characterized by progressive dementia and chorea (*1*). Although the site of the genetic defect was recently shown to be on chromosome 4 (*2*), the pathogenesis of the disease remains unknown. The most striking neuropathological feature is severe atrophy of the neostriatum, with marked neuronal loss and gliosis (*3*). This neuronal loss is accompanied by a reduction in neurochemical markers in spiny neurons, including substance P, enkephalins, and γ-aminobutyric acid (*4*). Golgi studies show morphologic abnormalities in dendrites of spiny neurons, but the dendrites of aspiny neurons appear to be less affected (*5*).

We previously reported that concentrations of somatostatin, a neurotransmitter contained in aspiny striatal neurons (*6*), are elevated in the striatum in HD (*7*). It was unclear whether this increase was due to selective sparing of somatostatin-containing neurons or of fibers, some of which may originate from outside the striatum. The histochemical method for visualizing nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) identifies a specific subset of aspiny striatal neurons (*8*) identical to those showing immunoreactivity for somatostatin and neuropeptide Y (*9*). We confirmed this correspondence of diaphorase activity with both somatostatin and neuropeptide Y immunoreactivity in human striatum (*10*). This reaction allows a rapid and reliable visualization of neurons, with intense staining of the entire cell soma and its arborizations. We now report that cells containing NADPH-d are selectively spared in the striatum in HD.

The clinical diagnosis of HD was confirmed by neuropathologic examination. Postmortem brain tissue from four HD patients (mean age, 56 years; range, 36 to 76 years) and six age-matched controls (mean age, 59; range, 36 to 78) was dissected fresh, and 1-cm-thick coronal blocks of tissue were removed from the head of the caudate nucleus (CN) at the caudato-putaminal junction and placed in 10 percent neutral buffered Formalin at 4°C. The interval between death and dissection was less than 12 hours (HD patients, 1.7 ± 0.7 hours and controls,

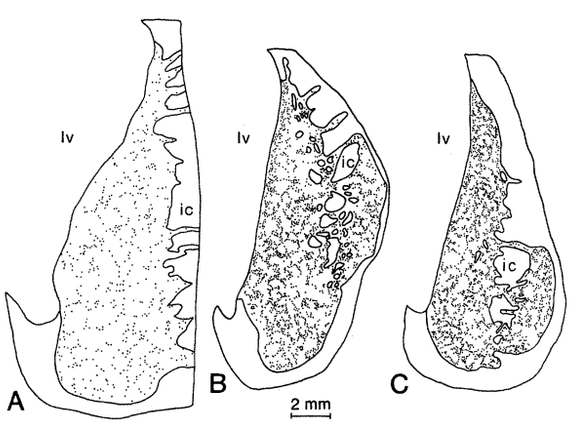

Fig. 1. Camera-lucida drawings of NADPH-d neurons (black dots), showing their distribution at the head of the CN in (A) a normal control and (B and C) two HD patients. NADPH-d neurons are distributed in a honeycomb pattern, forming bands around patches devoid of cellular staining. This pattern persists in HD, but the density of NADPH-d neurons is markedly increased. Abbreviations: ic, internal capsule and lv, lateral ventricle. Scale bar, 2 mm.

9.8 ± 1.0 hours; means ± standard errors). NADPH-d staining is stable for 24 hours in an animal model simulating human autopsy conditions (*11*). After being fixed for 24 to 72 hours, the tissue blocks were sectioned (100 μm) with a Vibratome and stained for NADPH-d by free-float incubation at 37°C for 1 to 3 hours (*12*). Selected sections were counterstained with cresyl violet for quantification of cell bodies.

Neurons were counted in sections at the level of the head of the CN (*13*). To assess the degree of neuronal depletion, 10-μm paraffin sections were stained with cresyl violet (*14*). The number of NADPH-d–positive neurons was counted in 100-μm Vibratome sections of CN. Percentages of NADPH-d cells were calculated by counterstaining 100-μm sections with cresyl violet and dividing the number of NADPH-d neurons by the total number of neurons per section (*15*). Surface area measurements of the CN were made with a Graf/Pen sonic digitizer in order to determine the density of NADPH-d neurons (*16*).

For biochemical measurements, tissue from 12 HD patients (mean age, 63; range, 16 to 69) and from 10 neurologically normal controls (mean age, 67; range, 35 to 87) were dissected as previously described (*17*). The diagnosis was confirmed in all 12 HD patients by neuropathologic examination. In all cases the time between death and refrigeration (4°C) was less than 24 hours. Intervals between death and dissection were 13.7 ± 3.1 hours for controls and 14.3 ± 2.6 hours for HD patients.

NADPH-d activity was determined by measuring the increase in absorbance at 580 nm resulting from the reduction of nitroblue tetrazolium (NBT) to diformazan products. Tissue (50 to 100 mg, wet weight) was homogenized by sonication in 0.5 ml of 50 m*M* tris buffer (*p*H 8.5) (*18*). The reaction was started by adding 200 μl of tissue extract to 2.0 ml of an assay mixture containing 0.55 mmol of NBT, 0.55 mmol of NADPH, 2.75 g of the detergent Cremophor EL (Sigma), and 55 mmol of tris buffer per liter. Blanks were obtained by boiling tissue for 10 minutes. The reaction was linear for up to 1 hour with tissue amounts of 50 to 100 mg. The reaction was run at room temperature and absorbance was measured at 1 hour. All results were analyzed with the Mann-Whitney *U* test and are expressed as means ± standard errors.

In both the HD and control groups, NADPH-d cells were evenly distributed throughout the CN in a honeycomb pattern. Cell-rich walls surrounded cell-poor areas (diameter, 0.5 to 2.0 mm) (Fig. 1). In tissue from HD patients, the walls were more cell-dense and the cell-poor areas were smaller. The CN was severely atrophic in all cases of HD. The medial surface, normally convex, was flat or concave and there was marked neuronal loss and gliosis. Despite the severity of tissue loss, there was a striking preservation of NADPH-d neurons (Figs. 1 and 2). Dendritic abnormalities of diaphorase cells were seen only in the most severe cases, in which the CN was reduced to a 3-mm ribbon. These abnormalities occurred in neurons adjacent to the ependyma and appeared as focal swellings or beading along dendritic arbors (Fig. 2).

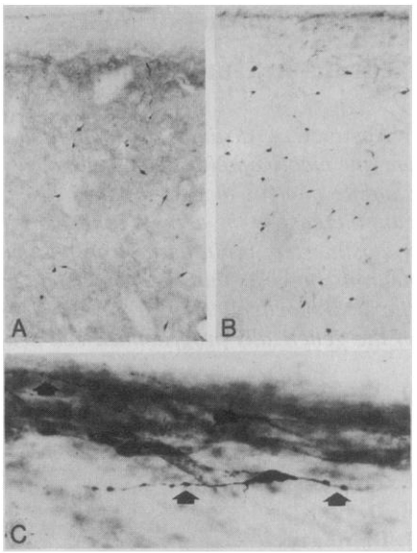

Fig. 2. Low-power micrographs showing NADPH-d cells (dark spots) in the CN adjacent to the ependyma in (A) a normal control and (B and C) HD patients. Dendritic beading (arrows in C) occurred only in the most severe cases of HD.

The total number of neurons in 10-μm sections at the head of the CN was significantly smaller in HD subjects than in controls (1010 ± 305 versus 4541 ± 130, $P < 0.01$), consistent with previous results (*3*, *19*). In contrast, the number of NADPH-d neurons in adjacent 100-μm sections was significantly larger in HD (1204 ± 168 versus 758 ± 45, $P < 0.025$), reflecting the combined effects of selective sparing of these neurons and tissue shrinkage in the section. The percentage of NADPH-d neurons was significantly larger in HD patients than in the controls (17.38 ± 6.01 percent versus 2.00 ± 0.08 percent, $P < 0.01$). The percentage was greatest in the most severe case (30.1 percent). In addition, the density of NADPH-d neurons in 100-μm sections of the CN was calculated and found to be significantly greater in HD striatum (1893.0 ± 14.6 versus 418.3 ± 25.2 cells per square centimeter, $P < 0.01$).

Biochemical measurements of NADPH-d activity confirmed the phenomenon of selective preservation of this enzyme in the striatum in HD: significantly higher levels were found in both the CN (2.90 ± 0.27 versus 2.02 ± 0.11 μmol/mg, $P < 0.025$) and the putamen (2.71 ± 0.26 versus 1.97 ± 0.14 μmol/mg, $P < 0.05$) (Fig. 3). There were no significant differences in frontal and occipital cortex. Biochemical differences in diaphorase activity were not as marked as differences in the density of NADPH-d neurons. Although NADPH-d neurons stain uniformly in tissue from HD patients, this may not indicate identical amounts of NADPH-d activity in every neuron.

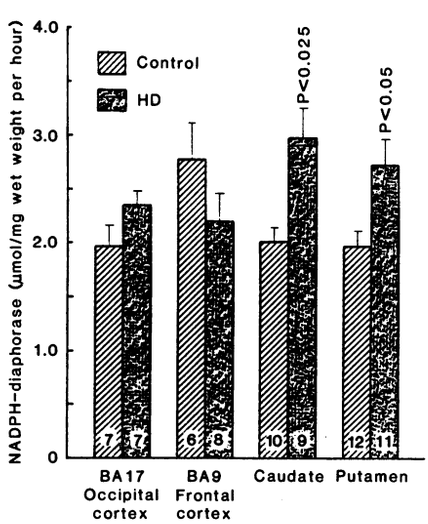

Fig. 3. NADPH-d activity in the cerebral cortex and striatum. The number of samples and the standard error of the mean are indicated on each bar.

Our results indicate that NADPH-d neurons are selectively preserved in the striatum in HD despite a severe loss of other neurons. A recent report (*20*) indicated that the density of somatostatin-immunoreactive neurons is proportional to tissue atrophy in HD and that fiber density is increased. Since neuronal density decreases in the striatum in HD, there may also be selective sparing of somatostatin neurons.

We previously found that concentrations of somatostatin-like immunoreactivity are three to five times higher than normal in the striatum in HD (*7*). This correlates closely with the approximately fivefold increase in the density of NADPH-d neurons found in the present study. Although we have not ruled out a contribution of extrinsic fibers to increased somatostatin concentrations, the increased neuronal density is sufficient to explain the findings.

Our findings support the conclusion that some biochemical feature of NADPH-d neurons makes them resistant to the degenerative process in HD. Diaphorase activity itself could be the responsible factor. NADPH-d participates in the detoxification of quinone derivatives (*21*). Excitotoxins have been used to model HD in animals, and one such neurotoxin, quinolinic acid (*22*), is present in the human brain (*23*) and produces selective neuronal lesions in rats (*22*). It is conceivable that the gene defect in HD could lead to accumulation of quinolinic acid or a similar compound. It has been shown in organotypic cultures that quinolinic acid toxicity depends on excitatory corticostriatal input (*24*). NADPH-d neurons might lack excitatory amino acid receptors, thus accounting for their resistance.

It is also possible that another as yet undiscovered property of NADPH-d neurons in the CN accounts for their viability in HD. Other unique properties of these neurons include their content of somatostatin and neuropeptide Y (*9*, *10*). Selective sparing of aspiny striatal neurons containing NADPH-d, somatostatin, and neuropeptide Y indicates that the neuronal loss in HD does not comprise a genetically programmed destruction of all cellular elements. These findings suggest that the gene defect is not expressed equally in all striatal neurons or is modified by the local biochemical environment.

Note added in proof. A recent report (*25*) indicates that both neuropeptide Y–like immunoreactivity and the number of neuropeptide Y–positive neurons are greater in the caudate nucleus and putamen in HD than in controls.

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13. One 10-μm paraffin section stained with cresyl violet and two 100-μm Vibratome sections, one stained for NADPH-d alone and the other for both NADPH-d and cresyl violet, were used in the cell counting.
14. All neurons with distinct nuclei and nucleoli were counted in 10-μm sections of the CN with a ruled-graticule eyepiece at 250×.
15. Due to the large number of total neurons, NADPH-d cells and total cells were counted along a 50-μm-wide track in the middle of the CN. The track was perpendicular to the ependymal surface and extended to the medial border of the internal capsule.
16. The area of the CN was measured from the ependyma to the medial border of the internal capsule at the level of the caudato-putaminal junction, excluding the nucleus accumbens.
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The 36-Kilodalton Substrate of pp60$^{v\text{-}src}$ Is Myristylated in a Transformation-Sensitive Manner

Abstract. *A primary intracellular substrate for pp60$^{v\text{-}src}$ kinase in a variety of avian and mammalian cells is a protein of 34 to 39 kilodaltons (kD). After incubation of chicken embryo fibroblasts (CEF) with [^{3}H]myristic acid for 4 hours, the 36-kD protein contained covalently bound myristic acid by several criteria: (i) the radioactively labeled material comigrated with the 36-kD protein on sodium dodecyl sulfate–polyacrylamide gels in one and two dimensions, (ii) the labeled material was insoluble in chloroform-methanol, and (iii) radioactively labeled myristate could be recovered from the purified 36-kD protein. The resistance of the acyl fatty acid moiety to hydrolysis by hydroxylamine suggested that the covalent linkage to the 36-kD protein may be through an amide linkage. The [^{3}H]myristic-acid labeling of the 36-kD protein in Rous sarcoma virus–transformed CEF showed a reduction of up to 45 percent when compared to an identical amount of 36-kD protein derived from normal cells; this reduction was not due to general changes in myristic acid metabolism in transformed cells.*

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Transforming proteins of several oncogenic viruses have tyrosine-specific protein kinase activity (*1*, *2*), and much effort has been expended to identify physiological substrates of this activity (*3*). One putative substrate is a protein of 36 kilodaltons (kD); in cultured cells this protein is phosphorylated on tyrosine in a transformation-dependent manner (*4–6*) and it can be phosphorylated in vitro on the same sites by pp60$^{v\text{-}src}$, the transforming protein of Rous sarcoma virus (RSV) (*5*). Recent reports have suggested that the 36-kD protein may have a structural (*7*, *8*) or signaling function (*9*) in epithelial cells. In eukaryotic cells some membrane-associated proteins, including the transforming protein of RSV (*10–14*), have covalently bound fatty acid (*15–18*).

To determine whether the 36-kD protein contains covalently bound fatty acid, both normal and RSV-transformed chicken embryo fibroblasts (CEF) were labeled in culture with [^{3}H]myristic acid for 4 hours and cellular extracts were immunoprecipitated with antiserum to the 36-kD protein. The specificity of the antiserum (*19*) was confirmed by comparison of the partial proteolytic maps of the immunoprecipitated 36-kD protein with published patterns and by the migration of the immunoprecipitated protein in a two-dimensional gel system (*5*). We found that cells incubated with [^{3}H]myristic acid contained a tritium-labeled 36-kD protein that was specifically immunoprecipitated by the antiserum (Fig. 1A). The [^{3}H]myristate in the 36-kD protein could be detected after an incubation period as short as 1 hour, suggesting that the labeled myristate may have remained intact. This tritium-labeled protein comigrated on two-dimensional polyacrylamide gels predominately with the more basic ($pI \approx 7.9$) and unphosphorylated form of the 36-kD protein in transformed cells (Fig. 1B). In normal cells, the 36-kD protein isolated by immunoprecipitation was not phosphorylated and the [^{3}H]myristate label comigrated with the entire [^{35}S]methio-