Inhibition in Huntington's Disease

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Huntington's disease (HD) is an autosomal dominant disease in which severe atrophy of the basal ganglia is accompanied by progressive dementia and chorea. An initial biochemical observation was that there was a marked deficiency of GABA and its biosynthetic enzyme glutamate decarboxylase (GAD) in HD basal ganglia. This was true of both the striatum and its sites of projection, the globus pallidus and substantia nigra. In our own studies we have confirmed the GABA deficiency and have shown that it correlates with pathologic grade. There is a gradient of GABA loss with the caudate being most severely affected followed by the putamen and nucleus accumbens. There were no significant changes in cerebral cortex. Studies of GABA receptors have shown reductions in the striatum with increased numbers of receptors in the pallidum, consistent with denervation hypersensitivity. Numerous trials of GABA replacement therapy using various agents have been unsuccessful despite evidence that these agents increase CSF concentrations of GABA. Therefore, the GABA deficiency alone is unlikely to be crucial for the clinical manifestations of HD. GABA deficiency appears to be a marker for loss of striatal spiny neurons in HD and knowledge of its deficiency has led to improved animal models of the disease.

Huntington's disease is an autosomal disorder of midlife onset characterized by progressive involuntary choreiform movements, psychological change, and dementia (Martin, 1984; Shoulson, 1984). Although there were several early descriptions of hereditary chorea the illness gained major recognition following the report of George Huntington in 1872. Huntington and his father had studied familes with the illness in East Hampton, Long Island. Although Huntington's disease can manifest itself either in children or the elderly the mean age of onset is typically 35–40. The movement disorder is the most conspicuous feature. The earliest choreic movements occur in the fingers, toes and face. These are quick jerky movements or sometimes a constant fidgeting. As the illness progresses the motoric disturbance spreads to the trunk and the oropharynx leading to progressive incoordination, immobility, unsteadiness, dysarthria and swallowing difficulties. Intellectual decline and dementia are invariable features of Huntington's disease. Emotional disturbances and changes in personality may precede or accompany the onset of chorea. Patients

with Huntington's disease are at high risk for suicide. The motor disturbances and dementia eventually lead to total incapacitation and patients often die after 10 to 25 years from inanition or pneumonia.

Neuropathology

The major neuropathologic findings in HD are a marked atrophy of the basal ganglia and frontal cortex. The extent of the neuronal degeneration found at the time of death is dependent on the length of time the clinical signs have been manifest and the age of onset. Vonsattel et al. (1985) have established a grading system from 0 to 4 for the degree of degeneration where 0 represents no visible alteration and grade 4 is severe atrophy of the basal ganglia. The microscopic changes typically show a loss of small and medium-size neurons in the basal ganglia with the relative proportion of glial cells increased (Lange, Thorner, Hopf, and Schroder, 1976). Vonsattel et al. (1985) found that the earliest pathologic changes were a loss of medium-sized spiny neurons in the most medial and dorsal portions of the caudate nucleus. Furthermore, Roos, Pruyt, deVries, and Bots (1985) described the greatest loss of neurons in the most dorsal regions of the putamen. The more ventral region of the caudate and putamen is contiguous with the nucleus accumbens, and it has been noted for some time that this limbic region of the brain rarely shows atrophy early in the disease. However, when there is severe brain atrophy, the nucleus accumbens may be reduced in size along with all other regions of the brain.

Striatal Anatomy

Nissl stained sections of striatum show two neuronal populations based on size. Medium-sized (10–20 micron) neurons account for 90% of the total and a large sized group makes up the remainder (Graybiel and Ragsdale, 1983). These neuronal groups can be further subdivided on the basis of Golgi studies into 5 subsets of neurons (Graveland, Williams, and DiFiglia, 1985). The majority (70–80%) of neurons are medium-sized spiny neurons (type I). These neurons are larger and have larger dendritic fields and lower spine density than their counterparts in the monkey (Graveland et al., 1985). A second spiny type (type II) is medium to large with sparse spiny dendrites (10%). Aspiny neurons are composed of medium (type I) and large (type 2) neurons. These neurons can be identified in Nissl stained sections by a characteristically indented nucleus. They have locally arborizing axons and account for 10–20% of human striatal neurons. The final category consists of small neurons with variable dendritic morphology (1–2%). Retrograde transport experiments have shown that the medium-sized spiny neurons account for most of the projecting neurons in the striatum while aspiny neurons are involved exclusively in local intrinsic striatal circuits (Graybiel and Ragsdale, 1983).

Several neurotransmitters have been localized to *spiny* type neurons. Spiny neurons contain the GABA synthetic enzyme, glutamic acid decarboxylase as well as GABA itself (Ribak, Vaughn, and Roberts, 1979). These neurons project to both segments of the pallidum and the substantia nigra pars reticulata (Van Den Pol, Smith, and Powell, 1985). Enkephalin is contained in about half of the GABA neurons (Aronin, DiFiglia, Graveland, Schwartz, and Wu, 1984; Penny, Afsharpour, and Kitai, 1986). These neurons project preferentially to the external pallidum and substantia nigra (DiFiglia, Aronin, and Martin, 1982b). Both substance P and dynorphin are also localized to striatal spiny neurons which project to the internal pallidum and substantia nigra pars reticulata (Bolam, Somogyi, Takagi, Fodor, and Smith, 1983; Chesselet and Graybiel, 1983; Zamir, Palcovits, Weber, Mezey, and Brownstein, 1984). Some substance P neurons also appear to contain glutamic acid decarboxylase (Penny et al., 1986).

Aspiny neurons contain a variety of neurotransmitters. Some neurons take up tritiated GABA and are presumed to be GABAergic (Bolam, Clarke, Smith, and Somogyi, 1983). The best characterized are medium-sized aspiny neurons in which both somatostatin and neuropeptide Y are colocalized (DiFiglia, Aronin, and Martin, 1982a; Kowall et al., 1987; Takagi, Somogyi, Somogyi, and Smith, 1983; Vincent and Johansson, 1983). The somatostatin-neuropeptide Y neurons account for 3–4 percent of the total striatal neuronal population. Of interest, these neurons also contain the histochemical marker NADPH-diaphorase. There is a 100% colocalization of these three neurochemical markers in human striatal neurons (Kowall et al., 1987). Both VIP and CCK have also recently been demonstrated in small populations of striatal aspiny neurons (Takagi et al., 1984; Theriault, Marshall, and Landis, 1984). Large aspiny neurons contain both choline acetyltransferase and the enzyme acetylcholinesterase (Vincent, Staines, and Fibiger, 1983).

Postmortem Studies of GABA in HD

Huntington's disease was one of the first degenerative illnesses in which postmortem biochemical measurements were made. Perry, Hansen, and Kloster (1973) were the first to report that levels of GABA are considerably reduced in the caudate, putamen, globus pallidus and substantia nigra in HD. They also found significant reductions of GABA in occipital and temporal cortex but not in frontal cortex or cerebellum. In their studies homocarnosine, a dipeptide consisting of gamma aminobutyrl histidine which is formed from GABA, was significantly reduced in caudate, putamen, globus pallidus and substantia nigra in HD. Increased concentrations of glycerophosphoethanol-amine in the same regions were unexplained.

These findings were confirmed in Bird and Iversen's study (1974) in which significant reductions in glutamic acid decarboxylase (GAD) activity and

GABA were found in HD caudate, putamen, globus pallidus and substantia nigra. In other brain regions such as frontal cortex, hypothalamus and hippocampus there was no significant decrease in GAD activity. The loss of GABA and GAD in the basal ganglia has been confirmed in several other studies (Ando, Gold, Bird, and Roth, 1979; McGeer, McGeer, and Fibiger, 1973; Stahl and Swanson, 1974; Urquhart, Perry, Hansen, and Kennedy, 1975).

Subsequently Wu (Wu, Bird, Chen, and Huang, 1979) and Spokes (1980) studied over 50 cases of HD and a comparable number of controls in which age and agonal status had been taken into account. They confirmed substantial reductions in GAD activity in the striato-nigral pathway. The activity of GABA transaminase, the enzyme which catabolizes GABA, was found to be normal in HD (Urquhart et al., 1975). As mentioned, homocarnosine, a metabolite of GABA, is reduced in HD, probably reflecting the GABA deficiency.

In our own studies we have measured concentrations of GABA in nine cortical and nine subcortical regions from 17 pathologically graded cases of Huntington's disease and ten controls without neurologic illness (Ellison, Beal, Mazurek, Malloy, and Martin, in press). We found that deficits of GABA correlated with increasing pathologic grades. There was a gradient of GABA loss across the striatal nuclei. The most marked changes were found in the caudate followed by the putamen and nucleus accumbens. Significant reductions in GABA content were found in both segments of the globus pallidus (external greater than internal) and both parts of the substantia nigra (reticulata greater than compacta). Measurements of GABA concentrations in cerebral cortex were normal in all nine regions examined including occipital cortex. We found no significant changes in hippocampus, claustrum, or subthalamic nucleus. An unexpected finding concerned a significant increase in GABA in the anterior nucleus of the thalamus but there was no alteration in the ventrolateral and dorsomedial thalamus. Interestingly, no significant alterations in taurine, another putative inhibitory neurotransmitter, were observed in any of the regions examined.

Consistent with the colocalization of GABA with enkephalin in striatal neurons, significant reductions in enkephalin have been found in HD globus pallidus and substantia nigra (Emson, Arrequi, Clement-Jones, Sandberg, and Rossor, 1980). Substance P may also be colocalized with GABA in some striatal neurons, and a large number of studies including our own have found reduced concentrations of substance P in HD (Beal, Ellison, Mazurek, Bird, and Martin, 1986; Beal and Martin, 1986).

CSF Measurements

Initial measurements of CSF concentrations of GABA in HD reported decreases. Perry and Hansen however argued that GABA in CSF was not

accurately measurable using previous techniques. Using refined techniques Perry et al. (1982) found no differences between CSF GABA in 73 controls and 22 HD patients. This has been confirmed in another study (Manyam and Tremblay, 1984). This finding suggests that basal ganglia GABA levels are not well reflected in CSF and that measurements will not be useful as an index of activity of disease.

GABA Receptors

Several tissue homogenate binding studies have shown reduced concentrations of GABA receptors in HD striatum and increased numbers of receptors in the substantia nigra (Lloyd, Dreksler, and Bird, 1977; Van Ness, Watkins, Bergman, Tourtellotte, and Olsen, 1982). Recent studies have employed quantitative autoradiography. Reductions in GABA receptors have been found in caudate and putamen as were increases in numbers of GABA receptors in the pallidum, consistent with a denervation hypersensitivity (Penney and Young, 1982; Walker, Young, Penney, Dovorini-Zis, and Shoulson, 1984).

GABA Replacement Therapy

The marked reductions of GABA in HD have led many investigators to attempt to restore GABA levels as a therapeutic approach to HD. Numerous attempts at GABA replacement therapy have been made with a wide variety of drugs. These have included muscimol (a GABA-mimetic drug) [Shoulson, Goldblatt, Charlton, and Joynt, 1978], isoniazid (a GABA aminotransferase inhibitor) [Manyam, Katz, Hare, Kaniefski, and Tremblay, 1984; McLean, 1984; Perry et al., 1982], THIP (a GABA receptor agonist) [Foster, Chase, Denaro, Hare, and Tamminga, 1983], and gamma acetylenic GABA (an irreversible inhibitor of GABA transaminase) [Scigliano et al., 1984; Tell et al., 1981]. Several of these agents were shown to elevate both CSF and brain GABA concentrations (Manyam and Tremblay, 1984; Perry, Wall, and Hansen, 1985; Tell et al., 1981). Despite this, all studies to date have shown no clinical improvement and no alteration in the course of the illness. These disappointing results suggest that a deficiency of GABA is not crucial to the clinical manifestations of HD.

Significance of GABA Deficiency in HD

Although the GABA deficiency in HD has not responded to replacement therapy it has stimulated further characterization of the neurochemical features of HD. The GABA deficiency most likely reflects loss of spiny neurons which utilize GABA as a neurotransmitter. As other neurotransmitter markers

of spiny neurons have been measured in HD, it has become apparent that they are similarly depleted. Thus, both substance P and dynorphin are depleted both in the striatum and its projection sites (Beal and Martin, 1986; Dawbarn et al., 1986; Seizinger et al., 1986). In contrast, neurotransmitters contained within aspiny neurons in HD are selectively preserved (Beal and Martin, 1986; Dawbarn, DeQuidt, and Emson, 1985; Ferrante et al., 1985). These include both somatostatin and neuropeptide Y. We have also recently found that acetylcholinesterase staining aspiny neurons are relatively preserved in the HD striatum (Ferrante, Kowall, Ross, Martin, and Richardson, 1986).

Many of these features can be reproduced in animals using the excitotoxin, quinolinic acid. We have found significant reductions of both GABA and substance P, yet somatostatin and neuropeptide Y are preserved (Beal, Kowall et al., 1986). Although the GABAergic deficit in HD may not be critical to the pathogenesis of HD, and probably is secondary to a pathologic process affecting all striatal spiny neurons, it has led to improved animal models of HD. Further studies of these models may improve our understanding of HD and could lead to therapeutic intervention aimed at halting the degenerative process.

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