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3-Hydroxyanthranilate oxygenase activity is increased in the brains of Huntington disease victims

(kynurenines/quinolinic acid/excitotoxins/neurodegenerative disorders)

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ABSTRACT **An excess of the tryptophan metabolite quinolinic acid in the brain has been hypothetically related to the pathogenesis of Huntington disease. Quinolinate's immediate biosynthetic enzyme, 3-hydroxyanthranilate oxygenase (EC 1.13.11.6), has now been detected in human brain tissue. The activity of 3-hydroxyanthranilate oxygenase is increased in Huntington disease brains as compared to control brains. The increment is particularly pronounced in the striatum, which is known to exhibit the most prominent nerve-cell loss in Huntington disease. Thus, the Huntington disease brain has a disproportionately high capability to produce the endogenous "excitotoxin" quinolinic acid. This finding may be of relevance for clinical, neuropathologic, and biochemical features associated with Huntington disease.**

Over the past several years, there has been remarkable progress in elucidating details of the genetic features of Huntington disease (HD) (1–3). However, the molecular mechanisms underlying the pathogenesis of selective neuronal loss in HD remain unidentified. One current hypothesis that has received considerable interest and investigative attention assumes that a pathological overabundance of an endogenous excitatory amino acid ("excitotoxin") in the brain is causally related to the occurrence of neurodegeneration in HD (4, 5). Of the excitotoxins considered so far, quinolinic acid figures most prominently. Quinolinate has been recognized for decades as a peripheral tryptophan metabolite (6, 7) and has recently been found to exist in brain tissue and human cerebrospinal fluid (5, 8). Lesions produced by intrastriatal injections of quinolinate in rats duplicate neuropathological and neurochemical changes that are characteristic for HD. For example, in both HD caudate and in quinolinate-injected rat striatum, there is a substantial reduction in the number of γ-aminobutyratergic (GABAergic) and cholinergic neurons but survival of medium-size Golgi type II neurons (containing somatostatin, neuropeptide Y, and NADPH diaphorase) and striatal afferents (5, 9–11).

The conversion of tryptophan to quinolinate via the kynurenine pathway (6, 7, 12, 13) has not so far been demonstrated in cerebral tissue (14). However, elements of the enzymatic machinery, including the biosynthetic enzyme 3-hydroxyanthranilate oxygenase [3-HAOxase; 3-hydroxyanthranilate:oxygen 3,4-oxidoreductase (decyclizing), EC 1.13.11.6] and the degradative enzyme quinolinate phosphoribosyltransferase [QPRTase; nicotinate-nucleotide:pyrophosphate phosphoribosyltransferase (carboxylating), EC 2.4.2.19], are present in the rat brain and can be expected to control the metabolism of quinolinate (15, 16). To date, only sporadic attention has been paid to the existence of the kynurenine pathway in the human brain. In addition to our recent report on QPRTase (17), we have now ascertained the presence and have examined the regional distribution of 3-HAOxase in brain tissue from patients who died without neurological disease and from individuals who died with HD.

MATERIALS AND METHODS

Brains used in this study were obtained from donations to the Brain Tissue Resource Center at McLean Hospital or from the Post-Mortem Brain Tissue Bank at Vanderbilt University. In all cases, brains were removed as rapidly as possible after death. Half (sagittal plane) of each brain was fixed in formalin for neuropathological evaluation. The other half was rapidly frozen, shipped on dry ice when necessary, and stored at −70°C. At the time of analysis, each brain was dissected and processed according to established procedures (18). Tissues were thawed at room temperature and sonicated (1:4, wt/vol) in distilled water. For 3-HAOxase measurement, 20 μl of the homogenate was incubated for 30 min at 37°C in 175 μl of 64 mM Mes [2-(*N*-morpholino)ethanesulfonic acid] buffer (pH 6.0) containing 0.3 mM Fe^{2+} and 7.2 μM (8.2 nCi; 1 nCi = 37 Bq) 3-hydroxy[*carboxyl*-^{14}C]anthranilic acid. The incubation was terminated by the addition of 50 μl of 6% $HClO_4$, and the product ([^{14}C]quinolinate) was isolated and measured as described (16). The stability of 3-HAOxase activity under similar storage and freezing/thawing conditions was ascertained in preliminary experiments with rat brain tissue (16). For glutamate decarboxylase determination, the original homogenate was further diluted (1:5) with 50 mM Tris·HCl buffer (pH 7.4) containing 0.2% (vol/vol) Triton X-100, and 25-μl samples were used for enzyme measurement (9). Protein content was determined in the original homogenate by the method of Lowry *et al.* (19) with bovine serum albumin as a standard.

Twenty-four control brains and 25 HD brains were used in the study. The demographic characteristics of the individuals studied (mean age, 64.6 years for controls and 67.8 years for HD patients; male/female ratio, 22/2 for controls and 12/13 for HD patients), as well as the intervals between occurrence of death and time of freezing of the tissue (mean, 5.5 hr for controls and 7.2 hr for HD patients), were recorded and considered for statistical comparisons of the two groups. HD brains were graded between 2 and 4 (average 2.7) according to the neuropathological scale of Vonsattel *et al.* (20).

RESULTS

3-HAOxase activity was clearly identifiable in normal human brain tissue and showed a pronounced and consistent re-

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Abbreviations, HD, Huntington disease; 3-HAOxase, 3-hydroxyanthranilate oxygenase; QPRTase, quinolinate phosphoribosyltransferase.

gional variability among 11 different brain regions studied. The enzymatic product, quinolinate, was identified in control caudate tissue in separate experiments using nonradioactive 3-hydroxyanthranilic acid as a substrate under otherwise standard assay conditions. The extent of conversion to quinolinate (determined radioenzymatically; ref. 21) was identical to that observed in routine radiochemical 3-HAOxase assays. In control brains, cerebral cortical regions contained one-fourth to one-sixth as much 3-HAOxase as the substantia nigra, the region with the highest enzyme activity (Table 1). Statistical analysis of control brain tissue revealed no significant correlation between caudate 3-HAOxase activity and the gender and age of the brain donor or postmortem interval (discriminate analysis and logistic regression). HD brains showed significant increases in 3-HAOxase activity in most brain regions. The increase in 3-HAOxase was found to be particularly pronounced in the striatum, reaching increments of +370% in the caudate nucleus and +360% in the putamen. No correlation was discerned between the neuropathological grade and the enzyme activity in caudate nuclei from HD brains.

Individual values of 3-HAOxase activity in caudate tissue are shown in Fig. 1*A*. Kinetic analyses, performed with the same tissues, indicated a substantial increase in maximal reaction velocity (V_{max}) in HD caudates (from 1.7 nmol per hr per mg of protein in controls to 7.8 nmol per hr per mg of protein in HD) and smaller changes in K_m values (Fig. 1*B*). In accord with published reports (22, 23), the activity of glutamate decarboxylase measured in HD caudates decreased by 61% as compared to controls [(from 21.0 ± 4.3 to 8.2 ± 1.6 nmol per hr per mg of protein (data are means ± SEM)].

DISCUSSION

In contrast to QPRTase, which exhibits approximately equal activities in rat and human brain (15, 17), 3-HAOxase activity on average was found to be about 2–3 times higher in human than in rat brain. The >100 times higher V_{max} value of 3-HAOxase as compared to QPRTase (17) gives rise to the question of *in vivo* regulation of quinolinate synthesis in the human brain. Thus, the anabolic enzyme 3-HAOxase can be expected to be under stringent regulatory influences at physiological conditions in order to avoid the rapid accumulation of quinolinate in either the intra- or the extracellular compartment. Control of product formation *in vivo* might be asserted by substrate availability, feedback mechanisms, or the direct modulation of 3-HAOxase by Fe(II) or other endogenous factors (16).

Table 1. 3-HAOxase activity in various brain regions

Region	Activity, pmol·hr^{-1}·mg^{-1}		Significance
	Control	HD	
Substantia nigra	2991 ± 522	4700 ± 637	0.040
Hypothalamus	2317 ± 430	3868 ± 747	0.074
Globus pallidus	2285 ± 374	4943 ± 765	0.003
Medulla	2217 ± 306	2804 ± 470	0.291
Caudate nucleus	1641 ± 293	5664 ± 755	<0.0001
Putamen	1420 ± 258	5093 ± 951	<0.0001
Cerebellum	1164 ± 205	2154 ± 334	0.014
Thalamus	1077 ± 125	2377 ± 347	0.001
Frontal cortex	782 ± 194	1685 ± 334	0.022
Hippocampus	740 ± 177	1315 ± 238	0.057
Parietal cortex	555 ± 90	1443 ± 348	0.018

Regions are listed in order of decreasing activity in the normal human brain. Enzyme activity (per mg of protein) was measured as described in ref. 16. Values are means ± SEM of 24 control and 25 HD brains. *P* values (significance) were determined by unequal-variance two-tailed *t* test with Satterthwaite's approximation for degrees of freedom.

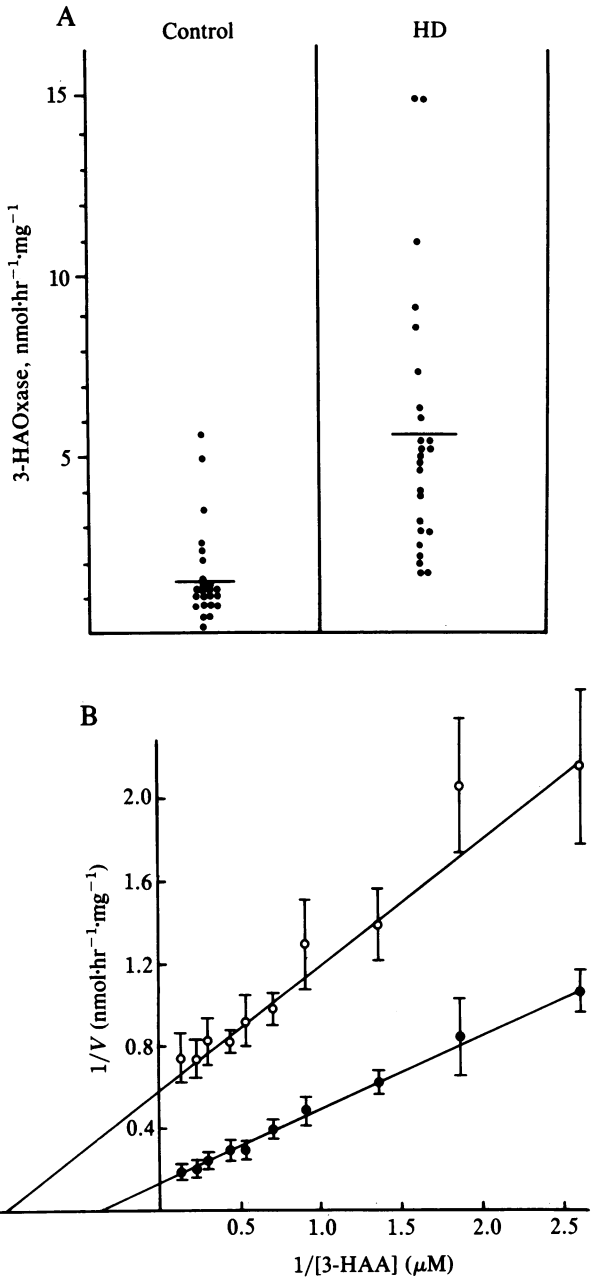

FIG. 1. (*A*) Individual values of 3-HAOxase activity (nmol per hr per mg of protein) in control and HD caudate nuclei. (*B*) Lineweaver–Burk analysis of 3-HAOxase activity in control (○) and HD (●) caudate tissue. Kinetics were investigated by varying the concentration of 3-hydroxyanthranilic acid (3-HAA) from 0.4 to 7.2 μM. Aliquots from 6–7 caudate tissues were pooled in order to include equal amounts of material from each of the 24 control and 25 HD brains. Each point on the curve reflects the mean ± SEM of the four control and HD pools thus obtained. Lines of best fit were obtained by linear regression analysis. K_m values were calculated to be 1.11 μM (control) and 2.86 μM (HD). V_{max} values are given in the text.

The results presented here indicate that 3-HAOxase in the human brain is predominantly localized in non-neuronal cells, since no decreases in enzyme activity were observed in brain regions that are known to be severely depleted of neurons in HD. This inference is in agreement with immunocytochemical studies of rat brain, where 3-HAOxase is found preferentially localized to astrocytic elements (24). Our findings also concur with data from rat striatal lesions, which indicate a severalfold increase in local 3-HAOxase activity

within days after experimentally induced loss of nerve cells (25). Thus, elevated levels of 3-HAOxase in the animal model are likely to be related to the proliferation of astrocytes containing this enzyme.

It is possible that the increase in 3-HAOxase activity in HD brains, too, is at least in part a reflection of the astrogliosis known to occur in diseased tissue (26). Thus, particularly pronounced enzyme changes were observed in the striatum. However, significant increases in 3-HAOxase activity also occurred in areas where there is less atrophy, and no correlation was found between the severity of neurodegeneration and the increments in 3-HAOxase activity. These findings indicate that unrecognized mechanisms of 3-HAOxase regulation might contribute to the enhanced ability of HD brains to produce quinolinate. Genetic factors may play a role; the idea of a genetically determined increase in the levels of an endogenous excitotoxin in the brain is concordant with recent discoveries on the molecular biology of HD (1–3). However, the lack of a significant change in 3-HAOxase activity in the medulla, for example, is not easy to reconcile with the notion that a genetic aberration related to 3-HAOxase function is responsible for the increase in 3-HAOxase activity described here; such an abnormality should probably be expressed equally in all brain cells.

The findings reported here provide evidence in support of the hitherto speculative link between quinolinate and HD. Unfortunately, nothing is known about the likely interdependence of brain 3-HAOxase activity and extracellular cerebrospinal fluid levels of quinolinate. Examination of the dynamics of this relationship *in vivo* seems to be of eminent importance given the excitotoxic potential of quinolinate. Thus, the measurement of quinolinate levels in tissue or in cerebrospinal fluid may not reflect the increased ability of HD brains to produce hyperphysiological and eventually toxic quantities of this compound. When the slowly progressive nerve-cell loss and associated clinical manifestations of HD (2, 5) are taken into account, only modest elevations of quinolinate levels—which possibly undergo intermittent variations and may be under the influence of nutritional factors—are to be expected. Such marginal increases are unlikely to be amenable to direct analytical assessment. Indeed, no changes in the concentration of quinolinate in urine (27) or in cerebrospinal fluid (28) have been detected in HD samples.

The demonstration of the human brain's ability to produce quinolinate also provides a basis for the investigation of the quinolinate system in neurological disorders other than HD. Since quinolinate is an agonist at the *N*-methyl-D-aspartate receptor (29, 30), a site that is suspected to play a role in the pathogenesis of several neurodegenerative conditions (31), a thorough examination of 3-HAOxase and other elements of the cerebral kynurenine pathway could reveal clues regarding the etiology of nerve-cell loss in temporal-lobe epilepsy, cerebral ischemia, hypoglycemia, or the normal aging process (32). Furthermore, it is possible that pharmacological agents that interfere with quinolinate production in the brain may be of therapeutic value for the prevention or arrest of the neurodegenerative process.

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