Positron Emission Tomography Measures of Benzodiazepine Receptors in Huntington's Disease

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We performed positron emission tomographic (PET) measurements of the regional distribution volume of benzodiazepine receptors and regional glucose metabolism in 6 drug-free patients with early Huntington's disease following injection of [^{11}C]flumazenil, a nonsubtype selective central benzodiazepine receptor antagonist, and ^{18}F-2-fluoro-2-deoxy-D-glucose, respectively. Flumazenil data were analyzed with a recently developed two-compartment, two-parameter tracer kinetic model. Benzodiazepine receptor density is related to distribution volume for flumazenil. In comparison with a group of healthy volunteers, benzodiazepine receptor density was significantly decreased in the caudate nucleus. Glucose metabolism was significantly reduced not only in the caudate nucleus but also in the putamen and thalamus. The changes in benzodiazepine receptor density observed in the caudate nucleus are commensurate with data obtained in postmortem autoradiographic studies of receptor density. Based on such postmortem studies we also anticipated changes in putamen and thalamic benzodiazepine receptor density. However, relatively little is known on receptor changes in early Huntington's disease, because the autoradiographic data available were obtained mostly in patients with advanced disease. The decreased glucose metabolism in the caudate and putamen agrees well with previously published results of PET studies, whereas metabolic impairment of the thalamus has not yet been described in Huntington's disease. The present study suggests that regional metabolism and γ-aminobutyric acid (GABA)–benzodiazepine receptor changes in subcortical structures of patients with early Huntington's disease do not occur with the same time course: Caudate benzodiazepine receptor density is already severely impaired when other subcortical structures reveal only minor abnormalities. Impairment of neuronal metabolism seems to predate GABA/benzodiazepine receptor changes since the putamen and thalamus demonstrate metabolic impairment without detectable loss of benzodiazepine receptor density.

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Huntington's disease (HD) is an autosomal dominant inherited disorder characterized by the onset in midlife of progressive motor and cognitive dysfunction. Previous studies with positron emission tomography (PET) and [^{18}F]-2-fluoro-2-deoxy-D-glucose (FDG) in patients with symptomatic HD have shown marked declines in caudate and putamen glucose metabolism early in the disease and possibly even presymptomatically [1–6]. The decline in caudate and putamen FDG uptake correlated with the decline in functional capacity of the HD patients. Over 90% of caudate and putamen neurons use γ-aminobutyric acid (GABA) as a neurotransmitter [7] and these neurons send projections to the lateral and medial globus pallidus and the substantia nigra pars reticulata. Postmortem biochemical studies have revealed large decreases in GABA concentrations and glutamate decarboxylase (GAD; the enzyme that synthesizes GABA) activity in HD caudate and putamen as well as their projection zones [8, 9]. In other postmortem studies of HD, binding sites for both GABA and benzodiazepines have been found to be decreased in striatum and increased in substantia nigra [9–14]. Postmortem autoradiographic studies of GABA receptors labeled with competitive GABA agonists and benzodiazepine agonists (that bind to a modulatory site on the GABA receptor) have shown decreased binding in caudate and putamen and increased binding in globus pallidus [15–18]. This latter increase presumably reflects a compensatory upregulation of pallidal receptors in response to denervation. In the superficial layer of the midfrontal cortex, total benzodiazepine receptor (BZR) binding was increased but its enhancement by GABA and barbiturates was significantly reduced [17]. We have now utilized the

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benzodiazepine antagonist [^{11}C]flumazenil (FMZ) to examine BZRs by PET in living HD patients, and compared the relative sensitivity of the method to measurements of glucose metabolism in the same subjects.

Materials and Methods

Subjects

Six patients (mean age, 53 ± 9 years; 3 men, 3 women) with adult-onset HD underwent neurological examination and evaluation of total functional capacity (TFC) according to the Shoulson and Fahn scale [19] prior to the PET studies. The diagnosis of HD was made on the basis of the family history, physical examination, laboratory tests to exclude other diseases, and the findings on computer tomographic (CT) or magnetic resonance imaging (MRI) scans. All patients studied here exhibited little or no cerebral atrophy as measured by cranial CT or MRI. TFC assesses the patient's ability to be gainfully employed, to handle financial affairs, to manage domestic responsibilities, to perform activities of daily life, and to be cared for at home. The TFC scale ranges from 13 to 0, with 13 being normal and 0 indicating total incapacitation. Patients were in stage I (n = 3) or stage II (n = 3), with a TFC for the group of 10.7 ± 1.6 (mean ± standard deviation). The estimated duration of symptoms was approximately 3 years in the stage I patients and 6 years in the stage II patients. They were compared with 6 normal control subjects (mean age, 50 ± 13 years) for the receptor studies and with 17 normal control subjects (mean age, 52 ± 10 years) for glucose metabolism studies. Control subjects were free of significant general medical, neurological, and psychiatric illness and had no family history of first-degree relatives with familial neurologic or psychiatric disease. They were also screened to exclude history of head trauma with loss of consciousness, drug or alcohol abuse, or dependence and excessive consumption of caffeine. They were free of prescribed sedatives, tranquilizers, and anxiolytics for 12 months prior to participation. The studies were approved by the University of Michigan Institutional Review Boards and written informed consent was obtained for all studies.

Positron Emission Tomography Imaging

A radial artery and cutaneous vein were cannulated for withdrawal of blood samples and for injection of the radioisotopes, respectively. The subjects were positioned with their eyes and ears unoccluded in the gantry of a CTI/Siemens 931/08-12 tomograph (Siemens Gammasonics, Inc, Hoffman Estates, IL). The scanner simultaneously acquires 15 contiguous transaxial slices covering approximately a 10-cm axial field of view, with an inplane spatial resolution of approximately 7- to 8-mm full width at half maximum.

The 6 patients were first studied following injection of 22.5 mCi [^{11}C]FMZ and then underwent a second PET study following injection of 10 mCi FDG. The radioisotope injections were separated by 100 minutes to allow decay and washout of residual isotope. Six age-matched control subjects underwent a single study following injection of 22.5 mCi [^{11}C]FMZ. Another 17 age-matched control subjects were examined with FDG only.

A dynamic series of PET data (15 frames) and 30 to 35 arterial blood samples were obtained over 60 minutes for the FMZ studies. Local cerebral metabolic rates for glucose (LCMRG) were calculated using a three-compartment model and a single-scan approximation with gray matter kinetic constants derived from normal subjects and a lumped constant of 0.54. A calculated attenuation correction was applied to all image reconstructions.

Correction of the Arterial Input Function for Flumazenil

The time-course of [^{11}C]FMZ following injection was determined in arterial plasma with the use of an NaI (En^{3}hance, New England Nuclear, Boston, MA) well counter. Thirteen samples for each study were processed by liquid chromatography using Sep-Pak C_{18} (Waters, Milford, MA) cartridges to separate authentic FMZ from labeled metabolites as described previously [20]. The arterial blood was centrifuged and the plasma was applied to the column. A metabolite and an FMZ fraction were then obtained for each sample by prewashing the column with 9 ml of a 35% methanol/sodium phosphate buffer solution and 5 ml of 100% methanol, respectively. The separation was corrected for loss of authentic FMZ with the metabolite fraction on the basis of [^{3}H]FMZ added as internal standard to each sample prior to processing. The ratio of [^{11}C]FMZ to [^{11}C]metabolites was determined in the well counter and the recovery of [^{3}H]FMZ was determined by liquid scintillation spectrometry. The arterial input function was then corrected for the metabolite fraction.

Realignment of the Images

Reconstructed images were realigned with the use of small molecular sieve beads as fiduciaries to correct for subject motion during and between scanning sessions. One bead was placed on the forehead and one behind each ear, and they stayed tightly attached to the scalp during the entire scanning period. Approximately 1 μl of the ligand preparation was pipetted onto each bead as follows: 6 μCi [^{11}C] per bead prior to a receptor study and 1.5 μCi [^{18}F] prior to an FDG study. Following reconstruction of the dynamic PET sequence, each bead was marked with a cursor on the 7.5- to 10.0-minute frame and the (x, y, z) coordinates were calculated based on the radioactivity distribution in the vicinity of the beads. This frame defined the standard orientation. An automated routine then located and calculated the (x, y, z) coordinates for each bead in the other 14 frames. These frames were then realigned to the orientation of the standard frame. With this method, it is possible to correct for the three translational and three rotational degrees of freedom. Inplane (x and y) translation error is smaller than 0.1 pixel (approximately 0.15 mm), the axial translation error (z) is smaller than 0.1 plane (approximately 0.5 mm), and the rotation errors (xy, xz, yz) are smaller than 0.2 degree. Good realignment was achieved in all studies.

Compartmental Analysis of [^{11}C]Flumazenil Transport and Distribution

The dynamic series of emission scans obtained from the receptor studies was analyzed together with the metabolite-corrected arterial input curve on the basis of a two-compartment, two-parameter model. The model consists of an intravascular compartment and a single tissue compart-

ment accounting for the combined tracer activity of free, nonspecific, and specific binding pools. The estimated parameters are the ligand transport from blood to brain, K_1 (milliliter of plasma/milliliter of brain/min) and the clearance rate from tissue back to blood, k_2 (min^{-1}). The ratio of these two parameters provides an estimate of the ligand's distribution volume (DV) in the brain, which is assumed to represent predominantly specific BZR binding. As shown previously [21], DV is linearly related to BZR density (B_{max}, $pmol^{-1}$ min^{-1}). Parametric images of K_1 and DV were generated for each study on a pixel-by-pixel basis using a weighted integral technique similar to that used for the cerebral blood flow studies.

Data Analysis

Regions of interest were determined on the K_1 images obtained from the FMZ study and were directly superimposed on the corresponding images of the DV maps and FDG scans. The regions included the frontal cortex, the occipital cortex, thalamus, caudate, putamen, cerebellar cortex, and the pons. Values from structures identified in both hemispheres were averaged for each subject.

Based on previously published autoradiographic studies [15–18] as well as PET measurements of glucose metabolism [1–6], we were primarily interested in changes in the caudate nucleus, as well as the putamen and the thalamus. An a priori hypothesis predicted decreases in caudate and putaminal DV and glucose metabolic rate. K_1, which is largely proportional to regional cerebral blood flow, was expected to show similar changes as seen for regional glucose metabolism. Due to either conflicting results on changes in thalamic glucose metabolism in HD [2, 3, 5, 6] or lack of previous reports on alterations in thalamic BZR distribution, it was difficult to anticipate any changes for that region. Paired Student's t tests were applied for comparison of regional mean values for transport rate and distribution volume for FMZ in the patient and control groups. The change in the metabolic rate of the caudate and putamen in the HD patients (expressed as the percentage of the mean of the control patients) was compared to the change in the FMZ DV in the same regions (also expressed as a percentage of the control means) by analysis of variance (ANOVA).

Results

The Table summarizes the regional values for radioligand transport (K_1) and BZR DV obtained for the [^{11}C]FMZ studies in both patient and control groups. K_1 in both groups varied slightly from region to region and was highest in the thalamus, intermediate in the visual cortex and putamen, and lowest in the frontal cortex and posterior fossa. In HD, K_1 in the caudate was reduced by 17% ($p = 0.02$) when comparing patient and control groups. Furthermore, K_1 values revealed a nonsignificant decrease in the putamen by 9%, whereas in all other regions analyzed they were slightly increased.

DV (milliliter of plasma/milliliter of tissue) varied about fivefold between regions (see Table). As previously observed [20], the highest receptor densities in patients and control subjects were found in the frontal and visual cortices, followed by the putamen, thalamus, and cerebellar cortex. The lowest values were demonstrated in the pons. Values of DV were lower in patients with HD than in control subjects in all supratentorial regions but the decrease reached significance only in the caudate (26% reduction, $p = 0.02$).

The FDG scans were carried out in the same HD patients as were the FMZ scans but they were compared to a different group of 17 age-matched normal control subjects. Glucose metabolism in the HD group was significantly decreased in the caudate and putamen by 47% and 41%, respectively ($p < 0.0001$), and also in the thalamus by 18% ($p = 0.001$). The comparison of regional ratios for LCMRG revealed a reduction in caudate metabolic rate (47%) that was more substantial than the decrease in FMZ DV (26%; $F(1,10) = 17.272$; $p = 0.002$).

Discussion

Assessment of BZR density in living humans is now possible through the use of radiolabeled ligands and dynamic PET [20–25]. Central-type BZRs are closely related to GABA receptors, and both receptors exist on a family of ligand-gated chloride channels. GABA is the most important inhibitory neurotransmitter in the brain and its inhibitory function is mediated by changes in the chloride conductance of the neuronal membrane. Benzodiazepines increase the frequency of chloride channel openings, while the duration of opening events and channel conductance are not altered (for a review, see [26, 27]).

[^{11}C]Flumazenil is a nonsubtype selective, central-type BZR antagonist with a high relative proportion of specific, receptor-mediated retention in the brain [22–24]. It is devoid of significant intrinsic pharmacological activity, but potently antagonizes the effects of benzodiazepines. As demonstrated recently, a two-compartment, two-parameter tracer kinetic model provides precise estimations of the kinetic behavior of [^{11}C]FMZ in the human brain [20, 21, 25] and makes it possible to obtain pixel-by-pixel maps of radioligand transport (K_1) and receptor density (DV). DV is considered to represent predominantly specific BZR binding. Allosteric interactions between GABA and BZRs have been described (agonist binding to one receptor increases agonist binding to the other [26]), but as an antagonist, FMZ in vitro binding has been shown to be unaffected by endogenous or added GABA or barbiturates [28, 29].

The pathology in HD is profound in the caudate and putamen but not all striatal neurons die as a consequence of the inherited gene defect. As many as 90% of striatal neurons are projection neurons, contain GABA, and are medium spiny neurons [7, 14]. GABAergic striatal output neurons appear to inhibit

Regional Means for {[11C]Flumazenil Transport Rate (K_1) and Distribution Volume (DV) in the Brain[a]

Regions	Patients		Control Subjects	
	K_1	DV	K_1	DV
Caudate	0.28 ± 0.03[b]	1.62 ± 0.14[b]	0.33 ± 0.04	2.19 ± 0.48
Putamen	0.33 ± 0.03	2.87 ± 0.27	0.36 ± 0.03	3.04 ± 0.51
Thalamus	0.41 ± 0.03	2.71 ± 0.25	0.39 ± 0.05	2.96 ± 0.41
Frontal cortex	0.31 ± 0.03	4.20 ± 0.62	0.30 ± 0.02	4.57 ± 0.75
Visual cortex	0.36 ± 0.06	4.99 ± 0.71	0.35 ± 0.05	5.74 ± 1.04
Cerebellum	0.33 ± 0.06	3.24 ± 0.37	0.32 ± 0.06	3.25 ± 0.30
Pons	0.35 ± 0.04	1.09 ± 0.14	0.31 ± 0.05	1.01 ± 0.10

[a]Data are means ± standard deviations, in milliliters of plasma/milliliter of tissue/min.
[b]$p = 0.02$ by two-tailed Student's t test.

GABAergic neurons in the globus pallidus and substantia nigra, which in turn project to the thalamus and brainstem (for review, see [30]). Somatostatin and acetylcholine interneurons are relatively spared and the brunt of the pathology falls on the GABAergic projection neurons. As shown in postmortem autoradiographic studies in patients with HD, the consequences of the loss of these projection neurons are caudate and putamen atrophy and compensatory upregulation of GABA receptors and BZRs [16] in the neurons of the denervated projection zones. In advanced cases of HD (>10 years' duration) GABA and BZR density are reduced by approximately 44 and 55%, respectively, in the caudate and putamen. GABA receptors are increased by about 106% and BZRs, by 72% in the lateral globus pallidus [18]. Although the exact cellular location of BZR measured autoradiographically is not known, this increase is believed to be an attempt of the deafferentated neurons to maintain homeostasis by increasing their functional response to a decreased synaptic input [16]. In early HD (stages I and II) only two postmortem cases have been examined and in those BZR density was reduced significantly by 44% in the putamen and increased by 58% in the lateral globus pallidus [16].

This is the first study of BZRs in living individuals with HD. PET measurements of BZR density and glucose metabolism were made in 6 patients with early HD (stages I and II) following injection of [^{11}C]FMZ and [^{18}F]FDG, respectively. We examined BZR DV in cortical as well as subcortical regions and our particular interest was directed toward possible changes in the metabolic rate and BZR density in the caudate, putamen, and thalamus when comparing patients to healthy control subjects. The PET technique is excellent for measuring metabolic and neurochemical parameters early in a disease process without confounding factors of drug effects or other illnesses. It would also have been of interest to examine pallidal BZRs in vivo and to verify the changes described in postmortem studies of advanced HD and also two brains with early HD [15–18]. One of the restrictions of the PET technique for in vivo studies of basal ganglia, however, is that accurate measurements of globus pallidus activity are not possible due to its small volume, scanner resolution, and difficulty discriminating between putamen and pallidum in our PET images. The globus pallidus is threefold smaller than the putamen, and its volume is approximately 40% below that required for a structure to be measured accurately with our scanner resolution [31].

We found that radioligand transport (K_1) and BZR DV in the patient group are significantly decreased only in the caudate nucleus and did not reach significance in the putamen and thalamus. Glucose metabolism, however, is significantly reduced in the caudate as well as in the putamen and thalamus. The present study reveals findings and results that corroborate some of the previously published observations in HD. The changes observed in BZR density and glucose metabolism in the caudate nucleus are commensurate with previously reported results of postmortem autoradiographic studies [15–18] and with PET measurements of glucose metabolism [1–6], respectively. It has been described that in early stages of HD, as in the patients presented here, caudate metabolism is affected more dramatically than is putamen metabolism [3] and pathological findings reveal that the disorder begins in the caudate nucleus and then progresses to the putamen [15]. Although the patients studied here exhibited little or no cerebral atrophy as measured by cranial CT or MRI, it should be considered that the changes found for the caudate nucleus may arise either by loss of binding sites and decreased neuronal function or by atrophy and partial volume artifact. The latter, however, is not supported by the fact that the change in K_1 is far smaller than the changes observed for DV and glucose metabolic rate. Changes reflecting merely cell loss would be expected to effect both transport rate and glucose metabolism to a similar degree. The

modest decrease in putaminal DV differs from postmortem autoradiographic results that have described a pronounced decrease in BZR density. However, postmortem data were found in patients with advanced HD, and only 2 with early HD have been studied so far [16]. It could be that in early HD the typical decrease in putaminal BZR density has not yet occurred. Walker and colleagues [16] reported that putaminal GABA and BZR levels in 2 patients with early HD (without obvious neuronal cell loss) were intermediate between those of control subjects and patients with advanced HD. The pallidal BZR DV was increased in these early cases almost to the level seen in advanced HD [16]. We also considered the possibility that our measured putamen values were artificially high because of partial volume averaging of the pallidum (which is expected to show increased receptor density) and insula that may be contributing activity to the putamen. We have performed simulation studies, however, that do not support this hypothesis. Computer-generated PET data sets were produced simulating the isotope distribution found in [^{11}C]FMZ receptor scans using a computerized digital brain phantom as described previously [32].

Thalamic glucose metabolism is significantly reduced, but measurements of K_1 and DV in the [^{11}C]FMZ scans show no significant change. Previous studies either have failed to show a significant change in thalamic metabolism [2, 5, 6] or have demonstrated increased metabolism [3]. Metabolic impairment of the thalamus in HD could be the result of selective neuronal degeneration. Dom and colleagues [33] demonstrated a 50% decrease in the small-cell population of the ventrolateral group of the thalamus with a preservation of the macroneuronal population. The small cells constitute 25 to 35% of the neuronal population in this region. Decreased thalamic BZR density was revealed previously in a postmortem autoradiographic study of advanced HD only [15] and in rats following striatal kainic acid lesions [34]. More advanced or severe striatal pathology may be necessary for the production of secondary thalamic receptor changes.

The present study suggests that regional metabolic and GABA/BZR changes in subcortical structures of HD patients do not occur with the same time course. Impairment of neuronal metabolism seems to predate GABA/BZR changes in stage I and II patients. Caudate glucose metabolism and BZR density are already severely impaired when other subcortical structures reveal only minor abnormalities. It will be important to determine the relationship of BZR changes to cognitive and motoric symptoms in this progressive disease. Possibly, the decline in BZR density might provide a more accurate estimate of disease progression than reductions in glucose metabolism.

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