Benzodiazepine receptor quantification in Huntington's disease with [^{123}I]iomazenil and SPECT

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Abstract

***Objectives*—Increasing evidence suggests that metabolic changes predate neuronal death in Huntington's disease and emission tomography methods (PET and SPECT) have shown changes in glucose consumption and receptor function in early and possibly even presymptomatic disease. Because the $GABA_A$-benzodiazepine receptor complex (BZR) is expressed on virtually all cerebral neurons BZR density images may be used to detect neuronal death. In this study the regional cerebral [^{123}I]iomazenil binding to BZR was determined in patients with Huntington's disease and normal controls by a steady state method and SPECT.**

***Methods*—Seven patients mildly to moderately affected by Huntington's disease and seven age matched controls were studied. Brain CT was performed on all subjects. In each subject two [^{123}I]iomazenil-SPECT measurements were acquired—one with and one without infusion of flumazenil. The affinity constant of flumazenil (Kd) was calculated from the paired distribution volumes (DV) and the free plasma flumazenil concentration. The distribution volume of [^{123}I]iomazenil in the unblocked condition (DV_0) reflects the ratio between BZR density and Kd.**

***Results*—Flumazenil Kd was similar in the Huntington's disease group and the control group (11.3 *v* 11.2 mM). For the Huntington's disease group a 31% reduction in striatal DV_0 (p=0.03) was found. In the cortical regions, DV_0 was similar in patients and in controls. In Huntington's disease, DV_0 correlated significantly with functional capacity (p=0.04) and chorea symptoms (p=0.02). The clinically least affected patients displayed DV_0s within the range of those of the control group (19–35 ml/ml).**

***Conclusions*—The finding of an unchanged Kd of flumazenil in patients indicates that the BZR is functionally intact in Huntington's disease. That is, the reduction in DV_0 for BZR represents a selective decrease in the number of striatal BZRs. DV_0 significantly correlated with functional loss and [^{123}I]iomazenil-SPECT could be an important tool for validation of the effect of future therapeutic strategies aimed at limiting oxidative stress and free radicals in Huntington's disease.**

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Huntington's disease is a midlife onset autosomal dominant disorder that is characterised clinically by progressive involuntary choreiform movements, cognitive decline, and emotional disturbances. From the onset of symptoms to death there is an average life span of 15 years.[1] The genetic mutation underlying the pathogenesis of Huntington's disease was identified in 1993.[2] The cause of cell death, however, still remains unknown. Studies in humans and animals suggest dysfunction of energy metabolism and oxidative stress in the events of the cell death cascade in Huntington's disease.[3 4] Whether dysfunction in energy metabolism and oxidative stress are primary events or merely secondary constituents of the cell death remains to be established.

Studies with PET and SPECT have the potential to evaluate energy metabolism and receptor binding parameters during the course of a disease process in the living human brain without confounding factors such as postmortem delay and end stage non-specific tissue changes. The benzodiazepine receptor (BZR) is allosterically linked to the $GABA_A$ receptor complex. This receptor complex is expressed on virtually all cortical and striatal neurons and on their axon terminals.[5 6] This means that mapping of the BZR reflects the amount of viable neural tissue. In early Huntington's disease BZR mapping may be more sensitive as a tool for measurement of degeneration than CT and MRI, because the loss of volume may be counterbalanced by glial cell replacement.

The aim of the present study was to calculate BZR binding parameters in patients with early Huntington's disease and in age matched controls, and to correlate these findings with their clinical presentation. We used steady state analysis and the benzodiazepine antagonist [^{123}I]iomazenil as the SPECT ligand. All subjects were studied twice—with and without infusion of unlabelled flumazenil, a BZR antagonist structurally closely related to iomazenil.

Subjects and methods

SUBJECTS

Two groups were studied: seven normal subjects (six men, one woman) with a mean age

of 41 years (range 30–51 years) and seven patients with Huntington's disease (four men, three women) with a mean age of 45 years (range 30–52 years). The study was conducted according to the Helsinki declaration and The central ethics committee had approved the study. All subjects gave their informed written consent.

The normal volunteers had no history of neurological or psychiatric disorders and claimed to be alcohol free, benzodiazepine naïve, and drug free. Neurological examination was normal in all cases. All normal volunteers had normal CT images.

All patients were admitted from the Chorea Huntington register at the Institute of Medical Genetics, University of Copenhagen. They had been identified on the basis of a family history of Huntington's disease and typical symptoms and the diagnosis was confirmed in all patients by mutation analysis. The mean duration of disease was 4 years (range 2–8), measured from the onset of involuntary movements. At the time of the study, five patients no medicine and two patients were receiving tetrabenazin (12.5 mg three times a day). Tetrabenazin impairs dopaminergic neurotransmission by depleting central monoamines. None of the patients had received benzodiazepines or barbiturates on a regular basis and all were benzodiazepine naive for at least 3 months before the study. Functional capacity was rated according to the Huntington's disease functional capacity scale (HDFCS) proposed by Shoulson and Fahn.[7] The HDFCS assesses the patient's ability to gainfully work, to handle financial affairs, to manage domestic chores, and to perform activities of daily life. The maximum score representing normal functioning is 13. Neurological symptoms were scored according to the "unified Huntington's disease rating scale" (UHDRS).[8] Chorea, dysarthria, ocular pursuit, and gait were rated on a scale from 0 to 4, where 0 represents normal function.

METHODS

The experimental procedures have previously been described.[9] For determination of the individual parameters of receptor binding, each volunteer was examined twice with an interval of at least 1 week. Intravenous catheters for blood sampling and [^{123}I]iomazenil administration, respectively, were placed in both cubital veins. One study was performed with unblocked receptors and the other aimed at 50% blockade of the receptors using constant infusion of non-radioactive flumazenil as previously reported.[9 10] In the unblocked condition, an intravenous bolus of 120 MBq [^{123}I]iomazenil was injected. In the partially blocked condition, intravenous infusion of flumazenil was started 135 minutes before injection of 240 Mbq [^{123}I]iomazenil, and the infusion of flumazenil was continued until the study had been completed.

Brain SPECT was performed using a Tomomatic 232 (Medimatic Ltd, Hellerup, Denmark), with performance characteristics as previously described.[11] Reconstruction was performed using filtered back projection, and a 32×32 reconstruction matrix. During the study period, phantom studies were performed to assess the cross calibration factor between the SPECT system and the well counter. The subjects were positioned in an identical manner at different scanning sessions by the use of adhesive tape markers of the orbitomeatal (OM) plane, a plane through the lateral canthus of the orbitus and the external opening of the ear. Data were obtained at the OM+48 mm and OM+88 mm levels. The acquisition protocol started with eight frames of 10 seconds followed by four frames of 1 minute each and five frames of 10 minutes each. From 90 minutes after injection until the end of the study session, the subjects were scanned every hour using 10 minute frames.

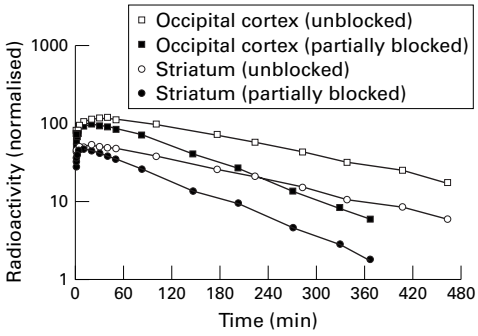

Figure 1 Time course of [^{123}I]iomazenil in striatum (circles) and occipital cortex (squares) in the unblocked condition (open symbols) and partially blocked condition (closed symbols) in patient 1. Radioactivity is normalised with injected dose in the unblocked and partially blocked condition, respectively.

Blood sampling for [^{123}I]iomazenil counting was done from the moment of injection, at 0–20, 20–40, and 40–60 seconds, and 1.5, 2, 3, 4, 5, 10, 20, 40, and 55 minutes. From 90 minutes after injection until the end of the study 7.5 hours after injection, blood samples were taken every hour. To derive the metabolite corrected plasma curve from the measured whole blood curve, octanol extraction of the parent compound (lipophilic) [^{123}I]iomazenil was done.[9] High performance liquid chromatography (HPLC) analysis of the plasma content of non-radioactive flumazenil was performed at 5, 120, 215, and 335 minutes. Plasma protein binding of flumazenil was determined by equilibrium dialysis.[9]

KINETIC ANALYSIS

The derivation of receptor indices was performed using the steady state approach of Lassen.[12] This method is based on calculating the distribution volumes of [^{123}I]iomazenil in the unblocked (DV_0) and partially blocked (DV_{pb}) situation.

$$DV_0 = \left[\frac{\int_0^\infty C_b(t)dt}{\int_0^\infty C_p(t)dt}\right]_0 \text{ and } DV_{pb} = \left[\frac{\int_0^\infty C_b(t)dt}{\int_0^\infty C_p(t)dt}\right]_{pb} \quad (1)$$

Distribution volumes were calculated from equation 1 using integrals of tissue curves, $C_b(t)$, and the metabolite corrected plasma curves, $C_p(t)$. After tracer injection the time activity curves (fig 1) were followed for 480 minutes in

Table 1 Characteristics of patients with Huntington's disease studied with [^{123}I]iomazenil, flumazenil, and SPECT

Patient No	*Sex*	*Age (y)*	*Duration (y)*	*Drugs*	*HDFCS (0–13)*	*Chorea (0–4)*	*Dysarthria (0–4)*	*Ocular pursuit (0–4)*	*Gait (0–4)*	*FH/CC*
1	M	49	5	No	6	4	3	1	1	1.57
2	M	37	4	No	10	1	1	1	1	1.67
3	F	51	8	No	4	3	3	1	3	1.43
4	M	52	2	Yes*	9	2	1	2	1	1.83
5	F	48	3	No	13	2	0	0	0	1.56
6	F	30	2	No	13	0	0	1	1	2.67
7	M	51	4	Yes*	5	3	2	3	3	1.54
		45 (SD 9)	4 (SD 2)							1.75 (0.42)

*Tetrabenazin (12.5 mg three times a day).

the unblocked condition and for 380 minutes in the partially blocked condition. The time activity curves were extrapolated to infinity using a single exponential determined from the last four data points. The fraction of the total area obtained by extrapolation was 13 (SD 2.2)% in the unblocked condition and 5 (SD 1.0)% in the partially blocked condition. The Kd of flumazenil was calculated from the paired distribution volumes, the average steady state plasma concentration of flumazenil, $C_p(L)$, the fractional free concentration of flumazenil in plasma, f_1, and the non-receptor-bound distribution volume of [^{123}I]iomazenil, λ, according to:

$$K_d = f_1 C_p(L) \frac{DV_{pb} - \lambda}{DV_0 - DV_{pb}} \quad (2)$$

For λ a value of 1 ml/ml was used.[9]

IMAGE ANALYSIS

Regions of interest (ROIs) were drawn on the individual CT images and transferred to the corresponding SPECT images for radioactivity and distribution volume measurements. The ROIs included the frontal cortex, the temporal cortex, the parietal cortex, the occipital cortex, and the striatum. The addition of ROIs defined in subsets of one of these regions (for example, frontal cortex) did not differ from that of the large region. This was also true for the added left and right sided regions.

Cerebral atrophy was evaluated using CT and the FH/CC ratio.[13] The FH/CC ratio is the ratio of the greatest distance between the frontal horns (FH) and the shortest distance between the medial surfaces of the caudate nuclei (CC). A trained radiologist blinded to the clinical status of the patients evaluated the CT. Unfortunately FH/CC ratios were only measured in three out of seven normal volunteers, as CT images were destroyed by accident after the ROIs were drawn.

STATISTICAL ANALYSIS

Regional cerebral [^{123}I]iomazenil binding was compared between patients and the controls using an unpaired two sample student's *t* test assuming equal variances. Correlations were calculated between regional DV_0 and FH/CC ratios and HDFCS and UHRDS test scores using Pearson's product moment correlation test. Statistical significance was set at $p<0.05$.

Table 2 Regional means (SD) for [^{123}I]iomazenil DV_0 and flumazenil Kd in patients with Huntington's disease (HD) and healthy controls

	HD		*Controls*	
Regions	*DV_0 (ml/ml)*	*Kd (mM)*	*DV_0 (ml/ml)*	*Kd (mM)*
Frontal cortex	40 (8)	10.4 (1.8)	41 (9)	11.0 (3.7)
Temporal cortex	38 (9)	10.6 (2.1)	41 (10)	11.7 (4.4)
Parietal cortex	38 (9)	10.4 (1.9)	40 (8)	11.0 (4.1)
Occipital cortex	45 (9)	11.0 (2.1)	48 (11)	11.7 (4.2)
Striatum	18 (5)*	11.3 (2.3)	26 (7)	11.2 (3.5)

*p=0.03 by two tailed Student's *t* test.

Results

Table 1 shows the clinical characteristics of the patients. The mean functional capacity score was 9 (range 4–13). The mean chorea score was 2 (range 0–4). The average FH/CC ratio in the Huntington's disease group was 1.75 (SD 0.42). The median FH/CC ratio in the three normal volunteers in whom FH/CC ratios were measured, was 2.57 (range 2.4–2.86).

The mean non-protein bound fraction of flumazenil was 0.62 (SD 0.01) and the mean free concentration of flumazenil was 13 (SD 3.03) nM.

Table 2 shows the Kd for [^{123}I]iomazenil DV_0 and flumazenil in the two groups. Flumazenil Kd in the Huntington's disease group ranged from 10.4–11.3 mM and did not differ significantly from the Kd of the controls in any region (range 11.0–11.7 mM). However, there was a trend towards a smaller Kd in the cortical regions of the patients. The striatum is the region most likely to be affected in early Huntington's disease. Here we could not demonstrate any difference in Kd between patients and controls. In fact mean Kd was slightly larger (0.1 mM) in the patients. The patients showed a significant (31%) reduction in striatal DV_0 (mean 18 (SD 5), range 12–25 ml/ml) compared to normal subjects (26 (SD 7), range 19–35 ml/ml, (p=0.03). The cortical DV_0s were similar in the two groups (table 2).

Figure 2 shows the correlations between striatal DV_0 and HDFCS, striatal DV_0 and chorea, striatal FH/CC and HDFCS, and FH/CC and chorea in the patients with Huntington's disease. Striatal DV_0 correlated significantly with both HDFCS (p=0.04) and chorea (p=0.02). There was no significant correlation between striatal DV_0 and dysarthria (p=0.09), ocular pursuit (p=0.08), or gait (p=0.09). A significant correlation between striatal FH/CC ratio and chorea was found (p=0.04), but there were no significant correlations between striatal FH/CC and HDFCS (p=0.14), dysarthria (p=0.15), ocular pursuit (p=0.89), or gait (p=0.51).

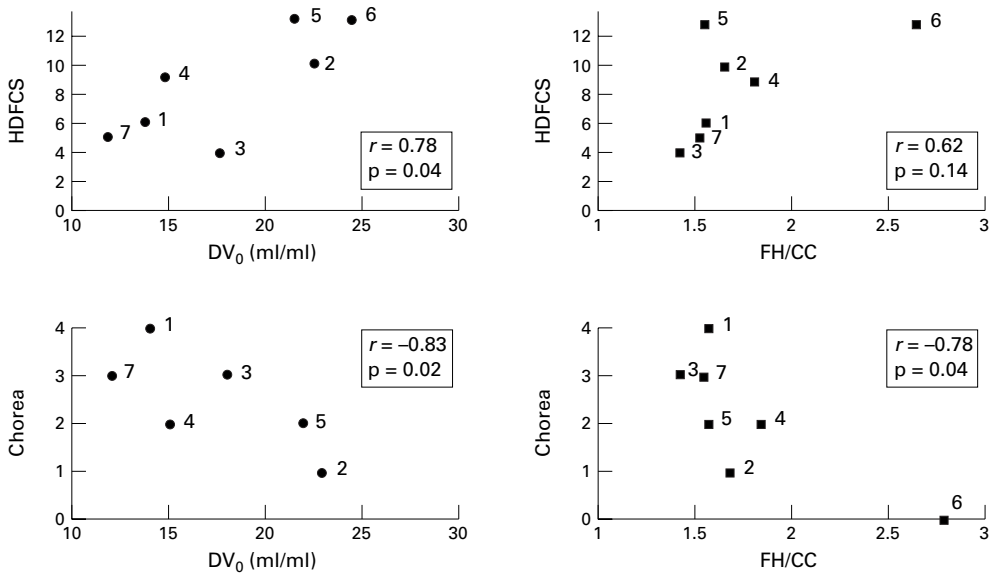

Figure 2 Plots of striatal DV_0 (reflecting receptor density) versus chorea and HDFCS, and FH/CC (reflecting central atrophy) versus chorea and HDFCS in the patients. Pearson's product moment correlation coefficient (r) and the probability (p) of no correlation tested against the t distribution are shown. Numbers correspond to numbers in table 1.

Discussion

In this study we showed an unchanged regional Kd for flumazenil in patients with Huntington's disease compared with controls. Consequently, a change in the availability for binding sites for the ligand is not accounted for by differences in the affinity between disease and control studies. Flumazenil Kd values correspond well with previously published values in normal subjects using SPECT and PET.[9 10] In these studies the Kd of the investigated ligand is usually assumed to be constant both regionally in the brain and between subjects. Such an assumption allows for performing only one study at high specific activity to obtain a measure of regional receptor density, as DV_0 can then be assumed to depend linearly on receptor density only.

We found a 31% significant reduction in striatal DV_0 in the patient group compared with the age matched control group. This agrees well with a 26% significant reduction in caudate DV_0 as previously shown using PET and [^{11}C]flumazenil in early Huntington's disease.[14] As we found the Kd of flumazenil to be unchanged in Huntington's disease it is concluded that changes in DV_0 reflect changes in BZR density. The three patients clinically least affected by the disease (patients 2, 5, and 6) had striatal DV_0 values within the lower range of the control group. The finding of striatal DV_0 within the lower normal range in patients with early Huntington's disease corresponds well with a report of quantitative autoradiography of BZRs in two patients with early Huntington's disease.[15] Contrary to the studies of BZR density, several previous studies with PET and [^{18}F]FDG have shown that caudate glucose metabolism in early Huntington's disease is below the 95% confidence limit of normal controls.[16–19] Hypometabolism has even been demonstrated in persons at risk for Huntington's disease.[16 18 19] Holthoff *et al*[14] studied patients with Huntington's disease mildly affected by disease progress with both [^{11}C]flumazenil-PET and [^{18}F]FDG-PET and demonstrated a reduction of 26% in caudate BZR density and a more substantial reduction of 47% in caudate glucose metabolism. We think that these PET and SPECT studies indicate that striatal metabolic dysfunction predates striatal BZR loss in Huntington's disease, and favours the hypothesis that metabolic dysfunction and oxidative stress are causative events in the cell death cascade.[3 4] We found no differences in cortical BZR DV_0 between mildly to moderately affected patients and the age matched control group (table 2), indicating that no or only minor loss of cortical synapses takes place at this stage of Huntington's disease. This is consistent with the PET study of Holthoff *et al* using [^{11}C]flumazenil.[14] In cortex glucose metabolism[16–21] and cerebral blood flow[22 23] data have been conflicting but tended to decrease only in late Huntington's disease.

In Huntington's disease it must be considered that changes in distribution volumes may arise either by loss of binding sites or by atrophy and partial volume averaging. We used the FH/CC ratio as a measure of striatal atrophy. In this study the average FH/CC ratio in the Huntington's disease group was 1.75 (SD 0.42). Although mild striatal atrophy was present in all patients except for patient 6, even in normal volunteers the FH/CC ratios varied considerably according to published FH/CC ratios (2.48 (SD 0.35) and 2.6 (SD 1.4)).[13 24] In our study, however, striatal ROIs were based upon individual CT images whereby the influence of striatal atrophy was minimised. That is, distribution volumes are considered primarily to represent receptor density. Furthermore, Kd values are even less influenced by atrophy and partial volume averaging as they are calculated as a ratio of distribution volumes in the unblocked and partially blocked situation.

In this study we demonstrated a significant correlation between striatal BZR DV_0 and

HDFCS and chorea (fig 1). A significant correlation was only shown between FH/CC and chorea (fig 1) Thus, in this small sample BZR DV_0 (BZR density) is a stronger predictor of functional and neurological decline in Huntington's disease than the FH/CC ratio. In future studies of BZRs in Huntington's disease it would be of interest to measure the actual ROI volume to calculate the ROI BZR number by multiplying the ROI BZR density with the ROI volume.[25 26]

In summary, in patients mildly to moderately affected by Huntington's disease the regional Kd for flumazenil was unchanged. On average there was a 31% reduction in BZR density and BZR density correlated well with clinical disease progress. The patients with Huntington's disease least affected by disease progress had striatal BZR densities within the lower range of the control group. This SPECT study of BZRs together with earlier [^{18}F]FDG-PET and [^{11}C]flumazenil studies in Huntington's disease suggest that changes in glucose metabolism predate BZR loss. Consequently, [^{18}F]FDG-PET is a more sensitive index for the early diagnosis and staging of Huntington's disease. However, changes in BZR density were found and correlated with functional capacity, which together with the lower cost and wider accessibility makes [^{123}I]iomazenil SPECT an important technique in the quantification of neuronal loss in Huntington's disease. Functional imaging is an important tool for future validation of the effect of neuroprotective strategies in neurodegenerative disease.

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