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SHORT COMMUNICATION

Normal glutamic acid decarboxylase activity in kidney tissue from patients with Huntington's Disease

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PERRY *et al.* (1973) demonstrated a reduction of gamma aminobutyric acid (GABA) in the striatum of patients with Huntington's Disease. MCGEER *et al.* (1973) found decreased glutamate decarboxylase (EC 4.1.1.15) activity in Huntington's Disease striatum, and this deficiency of GAD has been confirmed by BIRD & IVERSEN (1974), STAHL & SWANSON (1974), and MCGEER & MCGEER (1976*b*) in advanced cases of this disease. GLAESER *et al.* (1975) have correlated low cerebrospinal fluid GABA levels with Huntington's Disease. Although some authors (GILROY & MEYER, 1975) have concluded that the basic genetic–biochemical defect of Huntington's Disease is in the GAD/GABA system, this is not necessarily the case. Deficiencies of choline acetyltransferase, succinic dehydrogenase and dopamine have also been reported in brains of patients with Huntington's Disease (MCGEER *et al.*, 1973; BIRD & IVERSEN, 1974; STAHL & SWANSON, 1974). If the gene mutation of Huntington's Disease has a primary action on the GAD/GABA system, it is possible that its effects could be demonstrated in non-neural tissue since GAD and GABA are known to occur outside the CNS, especially in kidney (ZACHMANN *et al.*, 1966; HABER *et al.*, 1970*a*; LANCASTER *et al.*, 1973; LANCASTER *et al.*, 1975). GAD activity in rat kidney is about 15% of that in brain (MACDONNELL & GREENGARD, 1975). This present investigation evaluated GAD activity in kidney tissue from patients with Huntington's Disease and found no deficiency compared with controls.

MATERIALS AND METHODS

Post mortem studies were performed on four patients with histories, physical findings and family pedigrees typical of Huntington's Disease. Bodies were refrigerated at 4°C within 1-1/2 h of death in three patients and 3-1/2 h in the fourth (HD-2), and autopsies were performed within 24 h of death. Kidney cortex and brain tissue were dissected in a cold room (4°C) and were then frozen at −70°C until assayed. Neuropathological examination of one half of each brain confirmed the clinical diagnosis of Huntington's Disease. Assays on tissue from patient HD-1 were done between 5 and 8 months after death and within 1 month of death on patients HD-2, HD-3 and HD-4. Frozen kidney tissue from three additional patients with Huntington's Disease (HD-5, HD-6, HD-7) was provided by Dr. E. D. BIRD (Cambridge, England). This tissue was obtained under conditions previously reported (BIRD & IVERSEN, 1974). Times from death to assay of kidney tissue for these three cases were 5 months for HD-5 and 18 months for HD-6 and HD-7.

Post mortem tissue was also obtained from ten control patients without Huntington's Disease. No patients with renal disease or coma were included. Bodies of these control patients were refrigerated at 4°C within 2 h of death and autopsies were performed within 24 h of death [(30 h in patient C-3). (Quick refrigeration of tissue following death, rather than the delay in time to autopsy, has been reported to be the more important factor in determining enzyme levels in human brain and obtaining meaningful subcellular fractionation in guinea-pig brain (MCGEER & MCGEER, 1976*b*; SWANSON *et al.*, 1973)]. Dissected tissues were stored at −70°C until time of assay. Four control patients had both kidney and brain tissues assayed for GAD activity; in five patients only kidney tissue was assayed; and in one patient only brain tissue was assayed. Time of assay of tissues from control patients varied from 10 months post mortem for patient C-5 to 72 h post mortem for patient C-4.

Ten percent homogenates of kidney tissue were prepared in 0.32 M-sucrose (Tris buffer, pH 7.4) by initial homogenation for two periods of 4 s each at 60% speed in a Brinkmann Polytron (Brinkmann Instruments, Westbury, NY) followed by additional homogenation in a 10 ml Thomas Teflon–pyrex tissue grinder (two periods of 15 s each; 0.004–0.006 in clearance). Homogenates of brain tissue were prepared in a similar manner except homogenation in the Polytron was unnecessary.

GAD activity was measured by assaying GABA production using ion exchange resin separation of GABA from the reaction mixture (BAXTER, 1972). The micromethod used in this study was an adaptation of a technique provided by Dr. L. KREMZNER (Columbia University College of Physicians and Surgeons). L-[3-^{3}H]glutamic acid (23.4 mCi/mM) was purchased from New England Nuclear Corporation, Boston, MA. (No. NET-395; 0.0063 mg in 1 ml). The L-[^{3}H]glutamic acid was purified by first dissolving the sample in 6 ml of 0.01 M-acetic acid and adjusting to pH 8.5 with 0.1 M-NaOH. This solution was applied to a 4 ml column of 100–200 mesh Dowex-1 × 8 (which was previously treated with 10 vol of 1 M-acetic acid and washed with deionized water until the effluent pH was 4.9). Glutamate was absorbed on the column and the column was washed with 10 ml of H_2O with the non-absorbing material being discarded. The glutamic acid was eluted with 10 ml 0.3 M-acetic acid. The major peak contained in 3–4 ml of eluate was pooled and applied to a 3 ml column of 100–200 mesh Dowex 50 × 8 (which had been treated with 2 M-HCl and washed with deionized water until the effluent pH was 4.9). The column was washed with 10 ml H_2O and the eluate discarded. The glutamate was eluted

Abbreviations used: GAD, glutamic acid decarboxylase (EC 4.1.1.15); AOAA, aminooxyacetic acid.

with 15 ml 2 M-NH_4OH. Those fractions containing the major peak (about 3.5 ml) were pooled, lyophilized and redissolved in a small volume of 0.01 M-HCl.

GAD activity was measured as follows: To a disposable 6 × 50 mm micro test tube containing 10 μl of buffer (0.3 M-imidazole pH 6.5, 2 mM-2-aminoethylisothiouronium bromide, 1.5 mM pyridoxal-5'-phosphate) were added 2 μl of 0.3 M-L-glutamic acid, 8 μl of the purified L-[^{3}H]glutamic acid (containing approx 1 × 10^6 cpm) and 50 μl of the 10% kidney homogenate. The reaction was initiated by placing the tubes in a Dubnoff shaking water bath at 37°C, and stopped after 20 min by immediate immersion of the tubes in an ice bath followed by the addition of 50 μl of 0.5 mM-amino oxyacetic acid (AOAA). Next, 500 μl of deionized water was added to each tube and the contents pipetted onto a 0.4 ml Dowex 1 × 8 column (100–200 mesh, acetate form) contained in a standard pasteur capillary pipette. A second water wash of 500 μl was also added to the column. (Under these conditions it was demonstrated with known radioactive standards that greater than 90% of glutamic acid remained on the column and greater than 90% of GABA passed through the column.) The column eluate was mixed with 10 ml of Bray's scintillation cocktail (BRAY, 1960) and the [^{3}H]GABA determined in a Packard Liquid Scintillation Spectrometer. Results were expressed as μmol GABA formed/g wet wt./h. Within each assay duplicate or triplicate samples differed by 2%–10%, usually within 5%. The reaction product was shown to be GABA by TLC on silica gel in butanol–acetic acid–water (12:3:5). The same method was used for assay of GAD activity in brain tissue except 10 μl of homogenate were used and the imidazole buffer was pH 7.2.

RESULTS AND DISCUSSION

In kidney tissue from the controls the average GAD activity of 1.21 μmol of GABA formed/g wet/h wt. is similar to that reported by LANCASTER *et al.* (1975) in infants and children (but higher than the GAD activity in fetal kidney). In this study the average GAD activity in kidney tissue from control patients (1.21) was 29% of the average activity in the combined brain tissues of the controls (4.12). This study demonstrated no deficiency of GAD activity in kidney tissue from patients with Huntington's Disease when compared to controls (Table 1). There was a tendency for the values from the kidney tissue of the Huntington's Disease patients to be higher than the control values but this was not significant (*t* test of means, $P > 0.2$). Assays of GAD activity in the brain tissue from the patients with Huntington's Disease demonstrated less activity in the caudate and putamen than in the frontal cortex of each patient, consistent with previous reports (BIRD & IVERSEN, 1974; STAHL & SWANSON, 1974). This is unlike the usual finding in normal brain where GAD activity in the striatum often equals or exceeds that of frontal cortex, as shown in the control patients. However, as noted by other investigators (URQUHART *et al.*, 1975; FAHN, 1976), GAD activity in the striatum of control patients varies widely and may overlap the range of activity in the striatum of patients with Huntington's Disease.

The present finding of normal GAD activity in kidney from patients with Huntington's Disease supports the hypothesis that a deficiency in the GAD/GABA system is not the basic biochemical defect produced by the Huntington's gene. Although disordered brain GABA metabolism appears to be a consistent and important factor in Huntington's Disease, its relationship to the underlying genetic defect remains to be elucidated (MCGEER & MCGEER, 1976*a*). The characteristics of the striatum which make it particularly vulnerable to Huntington's Disease are unknown.

An important consideration bearing on the results of this study is the possibility that GAD from brain and kidney are different enzymes, and therefore, would not be

TABLE 1. GLUTAMIC ACID DECARBOXYLASE ACTIVITY IN KIDNEY AND BRAIN

Control patients			GAD Activity (μmol GABA formed/g/wet weight/h)			
Number	Sex	Age	Kidney	Frontal cortex	Caudate	Putamen
C-1	F	72	1.62	2.28	2.38	2.64
C-2	F	61	0.68	3.73	3.66	3.91
C-3	M	39	1.07	4.44	5.53	5.42
C-4	M	57	1.29	4.32	5.11	5.00
C-5	M	86	—	4.62	4.65	—
C-6	F	71	1.22	—	—	—
C-7	F	65	1.10	—	—	—
C-8	F	29	1.18	—	—	—
C-9	F	59	1.34	—	—	—
C-10	F	32	1.37	—	—	—
Mean		57.1	1.21 ±0.52(2 S.D.)	3.88	4.27	4.24
Huntington's Disease						
HD-1	F	41	0.99	3.78	0.53	1.81
HD-2	F	70	1.41	4.65	2.41	3.05
HD-3	F	47	1.70	2.60	1.16	1.80
HD-4	F	61	1.27	4.02	3.78	3.02
HD-5	F	41	1.16	—	—	—
HD-6	M	52	1.98	—	—	—
HD-7	M	71	1.28	—	—	—
Mean		54.7	1.40 ±0.68(2 S.D.)	3.76	1.97	2.42

expected to be equally affected by a single gene mutation. Several reports have indicated that the CNS contains primarily GAD-I and non-neural tissue contains primarily GAD-II, and these isoenzymes can be differentiated by response to anions, AOAA and pyridoxal phosphate (HABER *et al.*, 1970*a*,*b*). Arguments for and against the presence of two GAD isoenzymes are briefly reviewed by BAXTER (1972), MARTIN & MILLER (1976) and WU (1976). Unreported preliminary studies with the tissues used in the present project failed to show consistent differences between homogenates of kidney and brain in response to pyruvate, AOAA or pyridoxal phosphate. However, the question of differences in the GAD system between neural and non-neural tissue requires further investigation, particularly in regard to Huntington's Disease. If there are isoenzymes of GAD, it would be important to determine if only one is deficient in the striatum of patients with Huntington's Disease.

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