Homospecific activity (activity per enzyme protein) of tyrosine hydroxylase increases in Parkinsonian brain

Short Note


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Summary. Tyrosine hydroxylase (TH) contents in the caudate nucleus, putamen, and substantia nigra from control and parkinsonism brains were measured for the first time by a sandwich enzyme immunoassay. Both the TH protein content and TH activity ($V_{\text{max}}$) were decreased in parallel in the parkinsonian brains as compared with those of the control brains. In contrast, TH “homospecific activity” (activity per enzyme protein) was significantly increased in the parkinsonian brains. The results indicate that the decrease of TH activity in parkinsonian brains is due to the decrease of TH protein content as a result of cell death. The increase in the “homospecific activity” of residual TH in parkinsonian brain suggests such molecular changes in TH molecules as result in a compensatory increase in TH activity.

Keywords: Parkinson’s disease, tyrosine hydroxylase, homospecific activity, compensatory mechanisms.

Introduction

Tyrosine hydroxylase (TH) activity in parkinsonian brains decreases in nigrostriatal dopaminergic regions (Lloyd et al., 1975; Nagatsu, 1975; McGeer and McGeer, 1976; Nagatsu et al., 1977; Riederer et al., 1978). The decrease in TH activity is thought to be due to decreased TH protein following the cell loss of
the nigro-striatal dopaminergic neurons. On the other hand, the presence of inactive or less active forms of TH was observed by an immunoassay of TH protein in control human brains (Mogi et al., 1986). Although the physiological significance of the inactive TH protein remains to be further elucidated, increase in inactive forms of TH could also explain the decrease in TH activity in parkinsonian brains. There are also indirect evidences indicating some alteration of the property of TH in Parkinson’s disease. The decreases in striatal homovanillic acid (HVA) concentration were less severe than the corresponding loss in dopamine (DA) concentrations, suggesting a compensatory increase in transmitter turnover in the surviving dopaminergic neurons (Hornykiewicz, 1966). Similar increase in the ratio of HVA/DA was also reported in parkinsonian monkeys 2 months after administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Elsworth et al., 1987).

In the present study, we have measured both TH activity and TH protein using an enzyme immunoassay (EIA) (Mogi et al., 1984; Mogi et al., 1986), and have found increased “homospecific activity” (Rush et al., 1974; enzyme activity per enzyme protein) of TH in the nigro-striatal region of parkinsonian brains.

Materials and methods

Control human brains from 9 patients without neurological diseases and parkinsonian brains (7 cases) were obtained at autopsy in Juntendo University Hospital (Tokyo) and Ludwig Boltzmann Institute for Clinical Neurobiology (Vienna). The controls and parkinsonian patients were age matched (from 75 to 91 years). Postmortem times were from 4 hours to 10 hours. Caudate nucleus, putamen, and substantia nigra were dissected and stored frozen at —80°C. TH activity ($V_{max}$) and TH protein content were stable at —80°C.

Brain tissues were homogenized with 0.32 M sucrose, and the homogenate was centrifuged at 100,000 x g. The supernatant was used as the enzyme source.

TH activity ($V_{max}$) was measured by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) (Nagatsu et al., 1979). The incubation mixture (total volume 100 μl, in final concentrations) contained: 0.2 M acetate buffer (pH 6.0), 0.2 mM L-tyrosine, 1 mM (6RS)-methyl-tetrahydropterin with 100 mM mercaptoethanol, 10 μg catalase, 1 mM (NH₄)₂Fe(SO₄)₂, the enzyme preparation with 75 mM sucrose. The blank incubation contained 200 mM D-tyrosine instead of L-tyrosine. The reaction was carried out at 37°C for 10 min, and enzymatically formed DOPA was separated by double columns of Amberlite CG-50 and alumina, and measured by HPLC-ECD.

TH protein content in the enzyme samples were measured by an EIA using a monoclonal antibody against bovine adrenal medulla TH (Mogi et al., 1984; Mogi et al., 1986) and a standard TH protein purified from human adrenal medulla (Kojima et al., 1984). Preparation of the immuno reagents and the immunoassay system were essentially similar to those described previously (Mogi et al., 1984).

Results

Table 1 shows TH content (ng/mg of total protein), TH activity ($V_{max}$, pmol of dopa formed/min/mg of total protein), and TH “homospecific activity” (nmol of dopa formed/min/mg of enzyme protein) of the nigro-striatal region (caudate...