Quantification of mRNA of tyrosine hydroxylase and aromatic L-amino acid decarboxylase in the substantia nigra in Parkinson’s disease and schizophrenia

Rapid Communication

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Summary. Using the reverse transcription-polymerase chain reaction (RT-PCR), we developed a sensitive and quantitative method to detect all four types of human tyrosine hydroxylase (TH) mRNAs in the human brain (substantia nigra). All four types of TH mRNAs were found in the substantia nigra in the control brains examined, and the ratio of type-1, type-2, type-3, and type-4 mRNAs to the total amount of TH was 45, 52, 1.4, and 2.1%, respectively. The average amount of total TH mRNA in the normal brain (substantia nigra) was 5.5 amol of TH mRNA per μg of total RNA. The ratios of four TH isoforms were not altered significantly in Parkinson’s disease or schizophrenia. Further we measured the relative amount of aromatic L-amino acid decarboxylase (AADC) and β-actin mRNAs in the brain samples. TH and AADC mRNAs were highly correlated in the control cases. We found that parkinsonian brains had very low levels of all four TH isoforms and AADC mRNAs in the substantia nigra compared with control brains, while no significant differences were found between schizophrenic brains and normal ones. Since the decrease in AADC mRNA was comparable to that in TH mRNA, the alteration of TH in Parkinson’s disease would not be a primary event, but it would reflect the degeneration of dopaminergic neurons in the substantia nigra. This is the first reported measurement of mRNA contents of TH isoforms and AADC in Parkinson’s disease and schizophrenia.

Keywords: Tyrosine hydroxylase, aromatic L-amino acid decarboxylase, Parkinson’s disease, schizophrenia, RT-PCR, mRNA.
Introduction

Dopamine is one of the monoaminergic neurotransmitters, and it plays important roles in the brain functions such as emotion and behavior. Defective dopamine metabolism is associated with neuropsychiatric diseases, such as Parkinson's disease and schizophrenia. Dopamine is synthesized in vivo from tyrosine via 3,4-dihydroxyphenylalanine (dopa) by the sequential actions of tyrosine hydroxylase (EC 1.14.16.2; TH) (Nagatsu et al., 1964) and aromatic L-amino acid decarboxylase (EC 4.1.1.28; AADC) (Lovenberg et al., 1962).

TH is the rate-limiting enzyme for catecholamine biosynthesis (Levitt et al., 1965). Recent studies on the cDNA structure of human TH revealed the presence of four isoforms, designated as types 1 to 4 (Grima et al., 1987; Kaneda et al., 1987), which are produced by alternative splicing from a single gene (O'Malley et al., 1987; Kobayashi et al., 1988). These mRNAs differ from one another in having an inserted sequence consisting of 12 bp (type-2), 81 bp (type-3), and both 12 and 81 (93) bp (type-4) between nucleotides 90 and 91 of type-1 mRNA. However, a single species of TH mRNA corresponding to type-1 exists in the rat, mouse, and cow. Recently we demonstrated that New and Old World monkeys and the gorilla have only two TH isoforms, corresponding to type-1 and -2 of man (Ichinose et al., 1993). This finding showed that heterogeneity of TH increased progressively during the evolution of higher mammals. Generation of multiple TH isoforms in primates suggests the presence of an additional mechanism to regulate TH activity.

In Parkinson's disease, depletion of dopamine in the striatum by the selective destruction of nigro-striatal dopaminergic neurons causes dysfunction in motor-activity, i.e. hypokinesia and rigidity. Although the fundamental neurobiology underlying schizophrenia, one of the major psychiatric diseases, is unknown, alterations in dopaminergic neurotransmission have been suggested as possible factors in the pathogenesis of this disorder (Davis et al., 1991).

To examine the possible relationship of the expression of the dopamine-synthesizing enzymes to these diseases, we quantified mRNA contents of the four TH isoforms and AADC in postmortem human brains from control cases and patients with Parkinson's disease or schizophrenia.

Materials and methods

Brain specimens

Twelve adults (6 males and 6 females), who died neither of neurological nor of mental disorders, were used as control cases. Table 1 presents the mean age, the postmortem interval (the time lapse between death and autopsy), and sex distribution of the 12 control subjects, of 7 cases with Parkinson's disease, and of 8 cases with schizophrenia.

The diagnosis of Parkinson's disease was verified by neuropathological examination. Substantia nigra of all patients showed severe loss of neuromelanin. All these patients had been treated with anti-parkinson therapy (levodopa plus a peripheral decarboxylase inhibitor, 1-aminoadamantane and occasionally dopaminergic agonists).

The diagnosis of schizophrenia was verified by two research psychiatrists independently by a careful assessment of the clinical records of the patients. By using ICD-10 and DSM III R criteria, all patients belonged to the paranoid hallucinatory type of