Short Communication

Increase in the Level of mRNA for 3-Hydroxyanthranilate 3,4-Dioxygenase in Brain of Epilepsy-prone El Mice

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We had shown that production of quinolinic acid is high in the brain of epilepsy-prone El mice and that this is due to an increase in the activity of 3-hydroxyanthranilate 3,4-dioxygenase (3-HAO, EC 1.13.11.6). We demonstrated here that the level of mRNA for 3-HAO was markedly increased in the brain of El mice.

El mice have been used as a model for human genuine and hereditary epilepsy.1) A variety of studies have been done to explore the biochemical basis for the seizure susceptibility of these mice. For example, a defect of ceruloplasmin gene expression,2) high telencephalic expression of transcription factor AP1,3) and a deficit in the anticonvulsant endogenous methionine enkephalin4) have been observed in the brains of El mice. In addition, dysfunction of cerebellar glutamate receptors,5,6) and increased aspartate release from hippocampal neurons7) of the mice have been reported. Quinolinic acid (QUI), a natural brain constituent, is a structural analog of neurotransmitters such as L-glutamate and L-aspartate. Hence, it may act as an excitotoxin in the brain. Indeed, injection of a small amount of QUI into specific areas of the brain causes damage of neuronal cells and provokes seizures.8) These observations have led to a hypothesis that QUI is involved in the etiology of human neurodegenerative disorders such as Huntington's chorea and epilepsy. However, the concentration of QUI in the cerebrospinal fluid of epileptic patients is not higher than that in control subjects.9) We have shown that the level of QUI is high in the brain of epilepsy-prone El mice and that the increased production of QUI can be ascribed to an increase in the activity of 3-hydroxyanthranilate 3,4-dioxygenase (3-HAO, EC 1.13.11.6) in the brains of these mice.10,11) Eastman et al.12) have shown later that 3-HAO activity is markedly higher in both El mice and in DBA/2 mice, another seizure-prone strain, than two non-epileptic strains, BALB/c and Swiss-Webster. In this study we cloned and sequenced the cDNA for the enzyme and compared its expression in the brain of El mice with that of control ddY mice.

3-HAO was purified from the livers of male Sprague Dawley rats to a single band. Part of its amino acid sequence was analyzed after digestion with V8 protease. Oligonucleotides were designed in reference to the amino acid sequence and they were used as primers for reverse transcription-polymerase chain reaction of rat liver RNA. The resulting rat cDNA products were used to screen a rat liver cDNA library and to isolate a rat 3-HAO cDNA clone. Sequencing was done by the dideoxy chain-termination method. The results obtained showed that this clone has an insert of 1254 nucleotides and that the deduced primary structure of r3-HAO is composed of 286 amino acid residues (data not shown). The nucleotide sequence has been submitted to the DDBJ Data Bank with accession numbers D28339 and D44494. The rat amino acid sequence is very similar (87%) to the human 3-HAO.13) The similarity at the cDNA level was 76%. Total RNAs were extracted using the acid guanidium thiocyanate-phenol-chloroform (AGPC) method from the brains of El mice and of ddY mice, reverse-transcribed and amplified by PCR for 20 or 25 cycles. The primers used were 3-HAO sense and antisense. Amplified fragments were detected in the expected sizes by ethidium bromide staining or electrophoresed on 1% agarose gel and transferred to a Hybond N nylon membrane (Amersham). Hybridization was done with 3-HAO (1-1254) probe and _beta_ actin probe, respectively, which were labeled with [x-32P] dCTP using Multiprime.

![Fig. 3-HAO mRNA Expression in the Brain of Epilepsy-prone El Mice and of Control ddY Mice](image)

Two micrograms of their total cellular RNA was reverse-transcribed, and one-tenth of this was then amplified by PCR for 20 or 25 cycles and 10 cycles by using primers for 3-HAO and _beta_-actin, respectively. The expected sizes for 3-HAO and _beta_-actin are 863 bp and 730 bp (arrow heads), respectively. The amplified fragments were electrophoresed and transferred to a nylon membrane. Hybridization was done with 3-HAO probe and _beta_-actin probe, respectively, and the labeled DNA fragments were made visible with a Fuji Bioimage Analyzer BAS 2000.

Abbreviations: 3-HAO, 3-hydroxyanthranilate 3,4-dioxygenase; QUI, quinolinic acid.
DNA labelling systems (Amersham). The labeled DNA fragments were made visible with a Fuji Bioimage Analyzer BAS 2000.

As shown in Fig., the specific DNA band for 3-HAO was clearly amplified from RNA preparations obtained from the brain of El mice and control ddY mice. The degree of amplification was greater for El mice than ddY mice, indicating that expression of the 3-HAO gene was increased markedly in the brain of El mice.

The results obtained in this study suggest that expression of the 3-HAO gene is markedly increased in the brain of El mice. Alternatively, stability of its mRNA is increased, or both. These observations may be strengthened by the finding that El mice possess congenital abnormalities of tryptophan metabolism in the brain.14)

References