Expression of mRNA for Inducible NO Synthase in Human Brain

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We studied expression of inducible NO synthase gene in human brain under conditions of acute or chronic intoxication. Acute alcohol intoxication was accompanied by changes in enzyme expression in certain brain structures.

Key Words: *inducible NO synthase; mRNA expression; reverse transcription*

The NOergic system plays a role in the development of diseases, neurotoxic damage, and pathological dependence. Activity of this system was evaluated in a variety of biochemical and histochemical studies. Here we studied expression of mRNA for inducible NO synthase (iNOS) in human brain under conditions of acute and chronic intoxication.

MATERIALS AND METHODS

The brain was chosen as the object for total RNA isolation because of maximum number of expressed genes (about 4500) and minimum concentration of nucleases. The samples were obtained during forensic medical examination of subjects who have died a violent death.

Total RNA was isolated from 10 structures of human brain (frontal cortex, parietal cortex, occipital cortex, limbic cortex, cerebellar cortex, locus coeruleus, hippocampus, globus pallidus, caudate nucleus, and putamen) by the standard method with guanidine thiocyanate. RNA samples were used for reverse transcription-polymerase chain reaction (RT-PCR) [8]. The content of cDNA was estimated and normalized. PCR involved commercial primers (Klontekh) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene as a positive control. PCR was conducted using specific primers for the iNOS gene [9]. The content and quality of mRNA and PCR products were determined by the method of electrophoresis in agarose gel followed by ethidium bromide staining. The gels were photographed with a Nikon Coolpix 4500 camera in UV light at 300 nm.

RESULTS

We studied brain samples from men and women (24-86 years) dying from mechanical trauma (n=9) and mechanical asphyxia (hanging, n=3). The presence of alcohol and narcotic drugs in the blood and urine was estimated in forensic medical examination by the method of gas-liquid chromatography (Table 1). The diagnosis made during forensic medical examination was confirmed by histological findings.

Most studies of iNOS expression under various pathophysiological conditions and during ontogeny were performed with cultured cells or experimental animals. Therefore, extrapolation of these studies to human is difficult [3,7,10]. Moreover, RNA isolated from homogenates of morphologically and functionally different brain structures or mixture of brain samples from subjects who have died from various reasons cannot be considered as homogenous [2,9,11].

mRNA is unstable and after death degrades much more rapidly compared to DNA [4,12]. However, stor-

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age of organs and tissues in city morgues under standard conditions (4-18°C, relative humidity 98%) does not complicate the isolation of high-quality total mRNA (Fig. 1).

Amplification of cDNA from 12 brain samples with GAPDH primers yielded a DNA fragment, whose size corresponded to the expected value.

PCR with specific primers detected iNOS gene expression in brain samples from subjects who have died from acute intoxication with alcohol (B, D, I, J, K) and narcotic drugs (L). The degree of alcohol intoxication varied from mild (D, I, K) to severe (B, J). The presence of acute intoxication was confirmed by the results of morphological examination. We revealed alterative changes in the epithelium of proximal renal tubules and liver (granular dystrophy and steatosis), diffuse myocardial damage, and typical microcirculatory disturbances (venous and capillary plethora; stases; and edema of the myocardial stroma, adrenal medulla, lungs, and brain).

Expression of iNOS mRNA was not detected in brain tissue H (despite the presence of alcohol and morphine in the blood and urine). This subject had moderate alcohol and drug intoxication and died from mechanical asphyxia (hanging). The prevalence of proliferative changes in the examined organs served as a sign for chronic intoxication.

iNOS expression was detected only in the parietal cortex, limbic cortex, cerebellar cortex, locus coeruleus, caudate nucleus, and putamen (Table 2, Fig. 2). Our results and published data [2,5,10] contradict the notion that the isoform of iNOS is constantly present in most structures of the brain [9]. It can be hypothesized that expression of this isoform reflects adequate reaction of brain structures to acute intoxication. Previous studies showed that total NOS activity in the

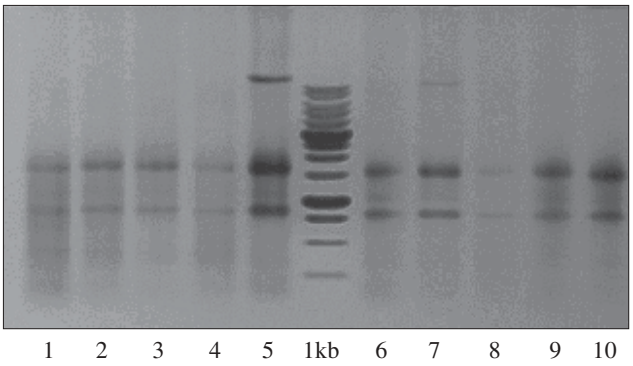

Fig. 1. RNA from brain H. Here and in Fig. 2: frontal cortex (*1*), parietal cortex (*2*), occipital cortex (*3*), limbic cortex (*4*), cerebellar cortex (*5*), locus coeruleus (*6*), hippocampus (*7*), globus pallidus (*8*), caudate nucleus (*9*), and putamen (*10*). 1 kb, DNA fragments with an increment of 1000 nucleotides.

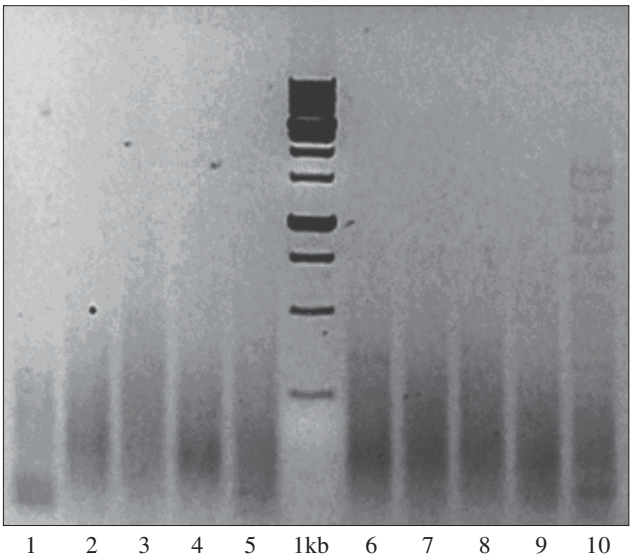

Fig. 2. PCR with specific primers for iNOS gene, cDNA of brain L.

TABLE 1. Results of Forensic Toxicology Study

Brain samples	Sex, age	Toxic compounds in the blood, urine, and tissues (‰)
A	Woman, 54 years	
B	Man, 41 years	2.74 alcohol (blood)
C	Man, 39 years	
D	Man, 60-65 years	0.5 alcohol (blood)
E	Woman, 86 years	
F	Man, 35 years	
G	Man, 26 years	
H	Man, 45-50 years	1.6 alcohol, 0.03 morphine (blood) 2.5 alcohol, 0.9 morphine (urine)
I	Man, 25-30 years	0.6 alcohol (urine)
J	Man, 50 years	4.7 alcohol (blood)
K	Man, 24 years	1.3 alcohol (blood)
L	Woman, 37 years	Morphine in bile, liver, and kidneys

TABLE 2. iNOS mRNA Expression in Structures of Human Brain

Human brain structure	A	B	C	D	E	F	G	H	I	J	K	L
Frontal cortex												
Parietal cortex									Detected			
Occipital cortex												
Limbic cortex										Detected		
Cerebellar cortex		Detected										
Locus coeruleus				Detected								
Hippocampus												
Globus pallidus												
Caudate nucleus				Detected							Detected	
Putamen										Detected		Detected

brain cortex significantly decreases after administration of ethanol [1]. It probably contributes to opposite changes in the nervous, glial, and vascular tissues of the brain in response to toxic exposure [1]. Therefore, NO plays a complex role in the compensatory and adaptive mechanisms.

Molecular and genetic studies demonstrate redistribution of iNOS in some structures of human brain. These data and results of standard biochemical and histological assays elucidate the role of the cerebral NOergic system in pathophysiological alterations (traumatic disease and intoxication).

Our findings can be used to develop a new approach of postmortem examination.

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