CONTENT OF NEURO-SPECIFIC AND NON-NEUROSPECIFIC ENOLASE ISOZYMES IN STRUCTURES OF THE HUMAN BRAIN

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KEY WORDS: neurospecific enolase; non-neurospecific enolase; brain; regional localization; immunoenzyme assay.

Disturbance of the enzyme systems of glycolysis has been discovered [1, 5] in patients with nervous and mental diseases. The comparative study of levels of some glycolytic enzymes in the brain in health and disease is therefore interesting.

One of the key stages of glycolysis, during which high-energy bonds are formed, is the conversion of 2-phosphoglycerate into phosphoenol pyruvate, catalyzed by an enolase that is present in the mammalian brain in two iso-forms: neurospecific (NSE) and non-neurospecific (NNE), located in neurons and glial cells, respectively [4, 8].

In the investigation described below NSE and NNE were assayed in different parts of the human brain. It is suggested that the results may be used for comparative evaluation of levels of these isozymes in the corresponding brain structures in psychopathology.

EXPERIMENTAL METHOD

Brains from persons dying suddenly from acute cardiovascular disease were used. Material was obtained not later than 5 h after death. The various brain structures and cortical areas were isolated by the standard method, using an atlas and cytoarchitectonic maps [2].

NSE was isolated from the human brain by the method described previously [3]. NNE was isolated from the fraction of principal water-soluble proteins (pH > 7.0), which was subjected to ion-exchange chromatography on carboxymethylcellulose CM-32 at pH 5.5, with elution of the bound proteins by a linear gradient of 0 to 0.5 M NaCl. The degree of purification of the enolase isozymes at each stage was studied by electrophoresis in polyacrylamide gel in the presence of sodium dodecylsulfate [3] and from the results of recording enzyme activity [7]. Antisera were prepared to both isozymes by the method described previously [3]. The antibodies were isolated from the antisera by immunoaffinity chromatography on Sepharose 4B, conjugated with a fraction enriched with the corresponding antigen. The specificity of the antibodies was tested in the double immunodiffusion agar gel test and by inhibition of enzyme activity. No crossed reaction was found.

To determine the concentration of the enolase isozymes, an immunoenzymic method was developed, using polystyrene plates (Nunc, Denmark), including the following stages: 1) 200 µl of the solution of antibodies in a concentration of 10 µg/ml in 0.01 M carbonate buffer, pH 9.5, was introduced into each well and incubated for 18-24 h at 4°C; 2) after washing, 200 µl of the solution of antigen in a concentration of 10-500 ng/ml and the same volume of solutions of the samples to be measured (concentration 5-50 µg/ml) in 1 mM potassium-phosphate buffer, pH 7.4, containing 0.05% Tween-20, was added and the mixture incubated for 2 h at 20°C; 3) 200 µl of a conjugate of the antibodies with horseradish peroxidase, made up with sodium periodate in a dilution of 1:250-1:1000, was added. After incubation for 3 h at 20°C 200 µl of reaction mixture (0.5 mg/ml of *ortho*-phenylenediamine and 0.005% H_2O_2 in 0.05 M citrate buffer, pH 4.8) was added to each well, the samples were incubated for 10 min at 20°C, after which 25 µl of a 0.5 M solution of sulfuric acid was added to each sample and optical density was measured at a wavelength of 492 nm. The sensitivity of the method was 5 ng/ml for NSE and

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25 ng/ml for NNE, due to differences in affinity of the antibodies. Differences between values obtained by measuring aliquots of the same sample did not exceed 15%.

EXPERIMENTAL RESULTS

The results of determination of NSE and NNE showed (Table 1) that the highest concentration of NSE (13-14 μg/mg) was found in the hypothalamic region (tuber cinereum), hippocampus, and limbic cortex, and also in some parts of the neocortex (12 μg/mg: frontal cortex — area 10, occipital cortex — area 17) and the lowest concentration (4 μg/mg) in structures of the brain stem. These findings are in good agreement with generally accepted ideas on the neuronal localization of NSE in the mammalian nervous system [3, 4]. Meanwhile a relatively low NSE concentration (6-7 μg/mg) was found in regions of the gray matter such as the cerebellar cortex, putamen, and parietal region of the cerebral cortex (areas 1, 2, and 3). This may be evidence of differences in the NSE concentration both in different types of neurons and also in neurons at different levels of functional activity.

The highest NNE concentration (34 μg/mg) was found in the thalamus. Since NNE in brain tissue is a specific marker for glial cells, it may be expected that the concentration of this isozyme is higher in structures rich in white matter than gray matter. Nevertheless, no relationship could be found between the NNE concentration and the relative proportions of gray and white matter in the structures studied.

The localization of enolase isozymes in the different structures of the human brain was studied for the first time. Similar investigations on the rat and monkey brain using radioimmunoassay have been conducted by Marangos et al. [6]. The general pattern of distribution of NSE was found to be similar in the rat, monkey, and human brain: the NSE concentration was high in structures rich in gray matter and low in structures of the brain stem. Differences in the NSE concentration in some structures of the monkey and human brain (a higher concentration in the hippocampus and hypothalamus in man, in the caudate nucleus in the monkey) may indicate interspecific differences. However, it must be recalled that these data (for the monkey's brain) were obtained by investigation of only one specimen.

It is envisaged that the data on the distribution of enolase isozymes in the human brain can be used for comparative study of the levels of these isozymes in brain structures of patients with mental diseases.

TABLE 1. Region of Distribution of Enolase Isozymes

Part of brain	NSE			NNE		
	n	$\bar{x}\pm\delta$	σ	n	$\bar{x}\pm\delta$	σ
Forebrain:						
frontal cortex (area 10)	10	12,4±1,5	2,1	6	24,8±6,2	6,5
temporal cortex:						
area 21	11	9,8±0,9	1,4	7	23,4±3,4	3,7
area 37	5	10,4±1,4	1,1	4	24,8±5,7	3,6
parietal cortex:						
areas 1, 2, and 3	11	7,2±1,2	1,8	11	22,6±2,3	3,4
area 7	3	10,4±5,0	2,0	3	23,8±2,0	1,1
occipital cortex (area 17)	9	12,2±1,8	2,4	3	21,3±6,1	3,3
insular cortex	10	11,2±1,3	1,8	4	22,5±10,5	6,6
limbic cortex	10	13,0±1,3	2,1	10	24,0±1,2	1,6
hippocampus	9	14,0±2,8	3,6	9	24,6±2,8	3,8
corpus callosum	8	6,9±0 9	1,1	2	28,8±11,7	1,3
caudate nucleus	5	11,0±1,5	1,2	2	25,1±6,3	0,7
putamen	7	5,6±1,9	2,1	—	—	—
globus pallidus	5	8,3±2,7	2,2	—	—	—
claustrum	3	11,0±1,3	0,7	—	—	—
Diencephalon:						
thalamus	5	10,6±3,5	2,8	3	34,4±5,7	3,2
tuber cinereum	6	14,0±5,1	4,9	3	19,4±11,2	6,1
Mesencephalon:						
corpora quadrigem	3	8,3±2,6	1,4	3	28,9±4,6	2,5
red nucleus	3	8,3±3,9	2,1	—	—	—
substantia nigra	2	5,3±2,7	0,3	3	25,4±7,9	4,3

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INTERACTION OF ISOLATED SYNAPTIC VESICLES WITH SYNAPTIC JUNCTIONAL COMPLEXES ISOLATED FROM THE RAT BRAIN

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KEY WORDS: synaptic vesicles; synaptic junctional complex; rat brain; Ca^{++} ions; calmodulin; exocytosis; turbidimetry.

The mechanisms (and methods of regulation under normal and pathological conditions) of secretion of mediators and hormones are under intensive study at the present time. Attempts to develop a model of exocytosis of mediators or hormones *in vitro*, essentially to study interaction of isolated secretory granules with the corresponding cell membranes, or more precisely, with the inner surface of the cell membranes of gland cells or with the presynaptic membrane (pre-SM), are consequently interesting [3]. During interaction between chromaffin granules (CG) and the fraction of adrenal cell membranes in the presence of Mg-ATP (phosphorylation substrate) and Ca^{++} (5 μM) liberation of labeled exogenous catecholamines and secretion satellites, namely ATP, dopamine-β-hydroxylase, and chromogranin [8], into the incubation medium, and also Ca-dependent phosphorylation of certain blocks of granules, and to a lesser degree, of plasmalemma proteins [9] are observed. Junction formation was recorded in these experiments by the sedimentation method: by the appearance of a new peak in the sucrose density gradient [10].

A similar approach has been used in relation to isolated brain synaptic vesicles (SV). It has been found [11], for instance, that interaction between SV and the synaptic membranes fraction of rat brain leads to acetylcholine release into the incubation medium. This effect can probably be explained by the formation of a junction between SV and the "everted" synaptosomal plasmatic vesicles present in the fraction used. Such interaction, induced by Mg-ATP, calmodulin (CaM) and Ca^{++} (1-5 μM) may lead to fusion of SV with the inner surface of the synaptolemma (with the pre-SM), as has been shown electron-microscopically [5].

The aim of this investigation was to study interaction of isolated SV with synaptic junctional complexes (the fraction consisting of the active zone of synapses) from rat brain by turbidimetry, a method not previously used. This method is basically suitable for recording the fusion (aggregation) of isolated SV with the pre-SM, a component of synaptic junctional complexes, quickly and over a period of time.

EXPERIMENTAL METHOD

The fraction of isolated SV was isolated from whole brain (without the cerebellum) of rats weighing 150-200 g bythe method described previously [2], suspended in 0.25 M sucrose, 20 mM Tris-HCl, pH 7.4, and used after a single freezing (−20°C) and thawing. The fraction of synaptic junctions (SJ) was obtained from whole rat brain (without the cerebellum) by the

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