Changes in Activity of Proline-Specific Peptidases in Rat Model for Dementia of Alzheimer's Type

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We studied the role of proline-specific peptidases in the pathogenesis of Alzheimer's disease. Testing of conditioned passive avoidance 24 h after learning showed that chronic administration of scopolamine to rats 4-fold reduced the latency of entry into the dark chamber in comparison with controls (intact animals). Activity of prolyl endopeptidase was significantly higher than in the controls in both the cortex and hippocampus. Changes in dipeptidyl peptidase IV activity were observed only in the cortex. Injection of AF-64A toxin into Meynert nucleus basalis reduced the latency of entry into the dark compartment by 75% in comparison with that in sham-operated and intact controls. Prolyl endopeptidase activity was reduced in the frontal cortex and hippocampus, but not in hypothalamus. Changes in dipeptidyl peptidase IV activity were detected only in the frontal cortex.

Key Words: *Alzheimer-type dementia*; *proline-specific peptidases; prolyl endopeptidase; dipeptidyl peptidase IV; enzyme activity*

According to WHO data, 35 million people worldwide are living with dementia of the Alzheimer type [5]. Cognitive disorders, including amnesia, lead to marked social disadaptation and sharply reduce quality of life in not only the patients, but also their relatives. Deciphering of the mechanisms of mental disorders and the search for new approaches to their pathogenetic therapy are important medical and biological and socio-economic problems. With growing number of patients with memory disorders caused by a variety of factors, health care spending rises [4].

There has been the recent considerable progress in understanding of the causes and pathogenesis of Alzheimer's disease (AD). Methods of laboratory diagnosis (identification of biomarkers of AD) and therapies are actively developed. Generally accepted markers of the disease are numerous senile plaques, extracellular spherical shaped amyloid deposits with polypeptide β-amyloid as the major component, and intraneuronal neurofibrillary tangles. Senile plaques forming near the degenerating axons and dendrites indicate neuronal death. AD is characterized by pronounced damage to the cholinergic system [12].

Contribution of neuropeptides to memory processes and its disorders attracted much recent attention [1,3,5]. Peptidases, proteolytic enzymes determining the quantitative and qualitative structure of regulatory peptides, are essential for manifestation of physiological properties of the peptide. Even minor malfunction of these enzymes can cause some CNS pathologies, including diseases associated with memory impairment. Many peptides mediating memory processes are known to be substrates for proline-specific peptidases, particularly of prolyl endopeptidase (PREP) and dipeptidyl peptidase IV (DPP IV).

Increased PREP activity was found in the brain of AD patients [13]. Changes in PREP activity and expression were revealed in memory disorders in experimental models of dementia of the Alzheimer type [14]. The data on PREP activity in these disorders are contradictory [2].

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There are no direct clinical or experimental data on DPP IV activity in memory disorders. However, endogenous peptides involved in the modulation of cognitive processes are known among substrates of the enzyme [11]. In this context, the involvement of these enzymes in the pathogenesis of AD is of special interest. This indicates the importance of studying the role of proline-specific peptidases and finding approaches to the development of new medicines based on their inhibitors [9].

MATERIALS AND METHODS

Experiments were conducted on male Wistar rats (n=210) weighing 180-200 g. The animals were kept under standard vivarium conditions with free access to food and water. The work was carried out according to the requirements of the European Union Council Directive on the use of animals for experimental purposes.

Chronic amnesia was modeled by intraperitoneal administration of scopolamine (Sigma) in a dose of 1.0 mg/kg for two weeks. The rats were trained on passive avoidance (PA) 24 h after the last introduction of scopolamine.

Animal model of Alzheimer-type dementia was produced by bilateral administration of choline toxin AF-64A in a dose of 57.5 μg on each side [7]. The dose of choline toxin AF-64A was previously determined. Intact animals served as the control, because PA behavior did not differ in intact and sham-operated rats [10].

PA training was performed in 2 weeks after surgery. The animals were decapitated after the behavioral experiments and the hypothalamus, hippocampus, and frontal cortex were isolated for biochemical studies.

Activity of proline-specific peptidases was measured fluorometrically. Protein content was assessed spectrophotometrically by the Bradford method [6].

Choline acetyltransferase (CAT) activity was measured by the method of Fonnum [8].

The data were statistically processed using Statistica 6.0 software by unpaired parametric (Student's t test) or nonparametric (Mann–Whitney U test) tests.

RESULTS

Passive avoidance testing 24 h after learning showed that chronic administration of scopolamine 4-fold reduced the latency of transition to the dark compartment in comparison with that in the controls (intact animals): 32.0±5.0 and 149.0±17.0 sec, respectively (p<0.01). Table 1 shows activities of peptidases and CAT in the frontal cortex and hippocampus.

Activity of CAT in the cortex was reduced by 20% and remained practically unchanged in the hippocampus. In experimental group, PREP activity was significantly higher in both the cortex and hippocampus. Changes in dipeptidyl peptidase IV activity were observed only in the cortex. Reduced CAT activity after chronic administration of scopolamine attests to the development of a condition that can be regarded as experimental model of Alzheimer-type dementia characterized by reduced activity of the cholinergic system.

Injection of AF-64A toxin into the Meynert nucleus shortened the latency of entry into the dark compartment by 75% in comparison with intact controls (29.0±6.0 *vs.* 112.0±12.0, respectively, p<0.01).

After bilateral injection of the toxin into the Meynert nuclei, CAT activity decreased in the frontal cortex (Table 2), increased in the hippocampus, and remained unchanged in the hypothalamus. Decreased prolyl endopeptidase activity was observed in the frontal cortex and hippocampus, but not in the hypothalamus. Dipeptidyl peptidase IV exhibited altered activity only in the frontal cortex.

These co-directed changes in proline-specific peptidase and CAT activities experimentally confirm that

TABLE 1. Enzyme Activity in Various Structures of the Brain of Intact Rats and Rats Treated with Scopolamine for 2 Weeks (***M*±*m***)

Enzyme	Activity, nmol×min^{-1}×mg^{-1} protein			
	frontal cortex		hippocampus	
	control (n=15)	scopolamine (n=9)	control (n=15)	scopolamine (n=9)
CAT	1.291±0.055	1.079±0.029*	1.514±0.037	1.519±0.020
PREP	0.485±0.010	0.512±0.005**	0.360±0.010	0.399±0.005**
DPP IV	0.093±0.005	0.075±0.003**	0.065±0.002	0.066±0.001

Note. Here and in Table 2: *p<0.05, **p<0.01 in comparison with the corresponding control (Student's t-test).

TABLE 2. Enzyme Activity in Various Structures of the Brain of Intact Rats and after Bilateral Injection of Toxin AF-64A into the Meynert Nuclei (*M±m*)

Structure		Activity, nmol×min^{-1}×mg^{-1} protein		
		CAT	PREP	DPP IV
Cortex	control (*n*=24)	1.302±0.093	0.472±0.022	0.069±0.003
	AF-64A (*n*=59)	1.010±0.057**	0.378±0.013**	0.056±0.003*
Hippocampus	control (*n*=24)	1.707±0.047	0.506±0.017	0.059±0.003
	AF-64A (*n*=59)	1.848±0.029**	0.426±0.015**	0.051±0.004
Hypothalamus	control (*n*=24)	0.931±0.044	0.352±0.016	0.327±0.010
	AF-64A (*n*=33)	1.019±0.051	0.058±0.003	0.051±0.002

both PREP and other proline-specific enzymes are involved in the pathogenesis of AD. These enzymes can thus be used as the target molecules for the development of new drugs.

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