CHANGES IN NEUROTRANSMITTER AMINO ACID LEVELS IN MOUSE BRAIN DURING SEIZURES INDUCED BY HYPERACTIVATION OF CHOLINERGIC STRUCTURES OF THE CNS

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Investigations have shown the importance of neurotransmitter amino acids for the development of seizure states [2, 5, 7, 8]. A comparative study has been undertaken of the time course of brain glutamate, taurine, and glycine levels in animals during convulsions induced by arecoline (a muscarinic cholinomimetic), nicotine (a nicotinic cholinomimetic), fluorostigmine (an acetylcholinesterase inhibitor), and picrotoxin (a substance with a noncholinergic mechanism of action).

EXPERIMENTAL METHOD

Experiments were carried out on 60 male albino mice weighing 16-20 g. The preparations were injected intraperitoneally in the following doses: arecoline 10 mg/kg, nicotine 9 mg/kg, fluorostigmine 3 mg/kg, and picrotoxin 8 mg/kg. The animals were decapitated 20 and 60 min after injection of the drugs. The brain tissue homogenate was extracted with a solution of 96° ethyl alcohol and 0.1 N hydrochloric acid in the ratio of 2:1. After centrifugation, the test amino acids in the supernatant were fractionated by thin-layer chromatography in medium consisting of: N-butanol—acetic acid—water in the ratio 4:1:1 [3]. After completion of chromatography (1.5 h), the chromatograms were developed for 10 min at 70°C with a 1% solution of ninhydrin in acetone, containing 10% acetic acid and 3% pyridine. The test amino acids were identified by the method of simultaneous application of substances with known Rf values: glutamate 28 mm, taurine 32 mm, and glycine 42 mm. After elution with acetone, amino acids were determined quantitatively by a spectrophotometric method (taurine and glycine at 420 nm, glutamate at 440 nm), followed by evaluation of the data with the aid of calibration curves. The results were subjected to statistical analysis with calculation of the arithmetic mean (M) and standard deviation (σ). The significance of differences between the values compared was judged by Student's t test.

EXPERIMENTAL RESULTS

Under these experimental conditions a seizure syndrome induced by any of the test substances was formed in the course of 5-15 min after their injection and lasted not more than 15-20 min. Against the background of seizures of whatever genesis, changes in the neurotransmitter amino acid levels were observed in the brain of the experimental animals (Table 1), in agreement with data obtained previously by Wade et al. [9]. The general principles of these changes can be clearly distinguished. For instance, the concentration of the excitatory amino acid glutamate rose rapidly during seizure formation (by 20-60% when determined after 20 min), after which its reserves became exhausted equally rapidly (60-20% of the normal level when determined after 60 min). The level of taurine, an amino acid with inhibitory neurotransmitter activity, rose (by 20-70% after 20 min) and remained virtually unchanged for at least 1 h. The concentration of the other inhibitory amino acid, glycine, in the early phase (20 min) either was unchanged or fell (by 50% after injection of nicotine), and then rose significantly (by 30-70%).

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TABLE 1. Effect of Arecoline (10 mg/kg), Nicotine (9 mg/kg), Fluorostigmine (3 mg/kg), and Picrotoxin (8 mg/kg) on Concentrations of Glutamate, Taurine, and Glycine in Albino Mouse Brain Tissues

Name of substance	Time after injection (in min)	Glutamate		Glycine		Taurine	
		mmoles/kg	%*	mmoles/kg	%*	mmoles/kg	%*
		$M \pm \sigma$					
Physiological saline (n = 6)	—	9,00±2,15	100	4,33±0,34	100	4,48±0,29	100
Arecoline (n = 12)	20	10,92±0,38	121	4,27±0,15	—	5,70±0,17	127
	60	10,25±1,14	—	3,90±0,30	90	5,40±0,67	121
Nicotine (n = 12)	20	8,75±1,18	—	2,3±0,12	53	5,48±0,37	122
	60	2,00±0,94	22	5,51±0,42	127	7,20±0,58	161
Fluorostigmine (n = 12)	20	14,0±1,22	156	4,28±0,48	—	7,72±1,08	172
	60	3,06±0,11	34	6,15±0,66	142	6,96±0,35	155
Picrotoxin (n = 12)	20	10,88±0,35	121	3,50±0,13	81	7,43±0,27	166
	60	5,51±0,45	61	7,44±0,56	172	6,20±0,26	138

Legend. Asterisk indicates that percentage was determined if differences from normal were statistically significant (probability of error $p < 0.05$).

Thus in the acute period of formation and extinction of the seizure syndrome two opposite processes were recorded: an initial rise of the level of the excitatory amino acid followed by exhaustion of its reserves and, at the same time, a gradual rise of the concentrations of inhibitory amino acids in the brain tissue.

Of the cholinergic drugs, this order of development of the response was most characteristic of the cholinesterase inhibitor fluorostigmine.

Nicotine had one distinctive feature of its action on glutamatergic structures, namely absence of the period of rise of the level of this amino acid in the brain tissue during nicotine seizures. Arecoline had virtually no effect on the glycine level. Incidentally this drug, in a higher dose (20 mg/kg) had a more substantial effect on glutamatergic and taurinergic mechanisms (the level of these amino acids rose respectively by 40 and 65% compared with 20 and 30% with a dose of 10 mg/kg). However, glycine was detected in this case in the same concentrations as in the tissue of intact animals.

These results indicate essential modulation of the state of the amino acid neurotransmitter mechanisms during hyperactivation of cholinergic structures of the CNS. This conclusion is in agreement with the view that close functional and morphological connections exist between the cholinergic and other neurotransmitter systems of the brain, and in particular, GABA-ergic [6], dopaminergic and noradrenergic [4], and so on.

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