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BINDING OF [^{3}H]-L-GLUTAMATE WITH SYNAPTIC MEMBRANES ISOLATED FROM THE CEREBRAL CORTEX AND HIPPOCAMPUS OF KRUSHINSKII—MOLODKINA RATS

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The appearance of epileptiform seizures in Krushinskii—Molodkina (KM) rats with a hereditary predisposition to audiogenic seizures is usually used as a model with which to study the inheritance and manifestations of human epilepsy. The biochemical mechanisms of the audiogenic seizure are considered to be based on disturbances of the mediator systems of the brain and, in particular, the glutamatergic systems [2]. Synaptic membranes isolated from the cortex, hippocampus, and certain other structures of the brain have been shown to be rich in high-affinity, stereochemically specific glutamate binding sites which, according to certain physiological and chemical-pharmacological characteristics, correspond to glutamate receptors [8]. Studies of the properties of membrane-bound receptors *in vitro* provide a biochemical approach to the evaluation of the state of the postsynaptic membrane, which determines the level of excitation of the nerve cell.

The aim of the present investigation was to compare binding of [^{3}H]glutamate with synaptic membranes isolated from various brain structures of KM rats at rest and after an audiogenic seizure.

EXPERIMENTAL METHOD

Experiments were carried out on adult male KM and Wistar rats weighing 200-250 g. The KM rats were obtained from the Laboratory of Genetics of Higher Nervous Activity, I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR.* Seizures were induced by application of an acoustic stimulus (60 dB, duration 5-10 sec). Synaptic membranes were isolated from the rats' cortex and hippocampus by differential centrifugation [10]. The protein content in the samples was determined by Lowry's method [7]. Binding of [^{3}H]-L-glutamate with the synaptic membranes was carried out as described in [3]. The membranes (50 µg protein) were incubated with 10-200 nM [^{3}H]-L-glutamate ("Izotop," USSR, 45 Ci/mmole) in 10 mM Tris-citrate buffer, pH 7.4, for 15 min at 37°C. The reaction was stopped by filtration through membrane filters (Synpor, Czechoslovakia, pore diameter 0.6 µ). The filters were washed with 2.5 ml of cold buffer. Nonspecific binding was determined in an incubation mixture containing 0.1 mM

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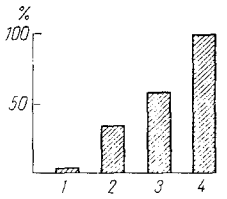

Fig. 1

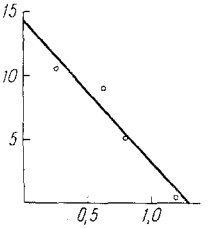

Fig. 2

Fig. 1. Binding of [^{3}H]-L-glutamate as a function of number of times synaptic membranes were washed. Abscissa, 1) fraction of synaptic membranes immediately after osmotic shock; 2, 3, 4) successive stages of washing membranes; ordinate, total binding of [^{3}H]-L-glutamate (in % of maximal).

Fig. 2. Binding of [^{3}H]-L-glutamate with synaptic membranes. Abscissa, specific binding (r, in pmoles/mg); ordinate, ratio of bound to free label (r/c, in fmoles/nM·mg).

TABLE 1. Binding of [^{3}H]-L-glutamate with Synaptic Membranes Isolated From Rat Brain

Test object	K_d, nM	B_{max}, pmoles/mg
Wistar rats		
cortex	84,0±22,30	1,11±0,13
hippocampus	97,7±18,80	1,15±0,22
KM rats		
at rest:		
cortex	89,8±18,10	1,23±0,09
hippocampus	91,8±23,60	1,22±0,21
during seizure:		
cortex	102,6±12,50	1,30±0,15
hippocampus	81,8±21,93	0,86±0,15

unlabeled L-glutamate. The filters were dried, dissolved in standard scintillation mixture containing Methylcellosolve, and radioactivity was determined with a Nuclear Chicago (USA) counter. The results were subjected to statistical analysis by a nonparametric method [1].

EXPERIMENTAL RESULTS

True receptor binding is considered to take place in the absence of Na^+ ions, whereas Na^+-dependent binding is ascribed to systems of active neurotransmitter transport [6]. Data on the dependence of specific Na^+-independent binding of [^{3}H]-L-glutamate on the number of times the synaptic membranes were washed in the course of their isolation are given in Fig. 1. An increase in specific binding presupposes the existence of an endogenous factor capable of inhibiting binding of [^{3}H]-L-glutamate. Preliminary experiments with fractionation of the eluate obtained during washing showed that the hypothetical inhibitor is not glutamic acid, is protein in nature, is thermostable, and actively inhibits binding of [^{3}H]-L-glutamate with synaptic membranes.

The results (Scatchard plot [9]) of determination of specific binding of [^{3}H]-L-glutamate with synaptic membranes isolated from the cortex and hippocampus of KM rats at rest are shown in Fig. 2. The linearity of the graphs indicates the presence of one type of receptor binding sites for [^{3}H]-L-glutamate in the brain structures studied. Values of the binding constant K_d and the concentration of binding sites B_{max} for KM rats, calculated from Scatchard plots (Table 1) are several times smaller than those given in the literature for Sprague-Dawley rats [5]. This can probably be explained by the milder method of obtaining synaptic membranes. Exclusion of the stage of ultrasonic treatment of the membrane preparation probably led to less complete separation of the binding inhibitor and, correspondingly, to a

reduction in the concentration of binding sites (B_{max}). The simultaneous decrease in the binding constant K_d, characterizing the affinity of the receptor for the ligand, is evidence that in this method of isolation the native structure of the receptor is less affected.

The experimental comparison of the parameters [^{3}H]-L-glutamate binding by cortical and hippocampal synaptic membranes from KM and Wistar rats in a state of quiet wakefulness showed that they do not differ statistically significantly. After an audiogenic seizure the affinity of the receptor for glutamate was virtually unchanged (Table 1), whereas the number of receptors was significantly (by 30%) reduced in the hippocampus. This was probably due to the high lability of the exciting receptors, in agreement with the view that the glutamate receptors of the hippocampus belong to the type of membrane receptors whose number may vary depending on the level of neuronal activity [4]. The decrease in the number of exciting receptors in the hippocampus after an audiogenic seizure and the absence of analogous changes in the cortex indicate the specificity of the reaction and is in harmony with views linking audiogenic seizures with subcortical structures [2].

The results suggest that glutamatergic pathways and, in particular, glutamate receptors are involved in the mechanism of spread of audiogenic seizure activity. Meanwhile the absence of statistically significant differences between the characteristics of binding of [^{3}H]-L-glutamate by the synaptic membranes of KM and Wistar rats in a state of rest indicates that the possible hereditary disturbances must be sought at the level of dynamic receptor activation.

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