EFFECT OF ANTAGONISTS OF EXCITATORY AMINO ACIDS ON GLUTAMATE RECEPTORS OF THE LOCUST AND SEIZURES INDUCED BY GLUTAMATE, ASPARTATE, KYNURENIN, AND QUINOLINIC ACID IN MICE

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In recent years quinolinic acid (QA) and kynurenin (KU) have attracted attention as neuroactive substances [3] belonging to the group of endogenous stimulators and convulsants [5]. Their neuroactivity has been established on insects [6], amphibians [4], and mammals [3, 5].

The aims of the present investigations were as follows: 1) to attempt to elucidate the structures with which excitatory amino acids (aspartic and glutamic acids, QA, and EU) interact on selected objects, using antagonists with known chemical structure; 2) to determine whether QA and KU, whose structure is similar to that of aspartic acid, especially as regards the distance between the carboxyl groups, evoked electrophysiological responses similar to responses to aspartate and glutamate, in the course of their action on glutamate receptors of locust muscle fibers; 3) to compare the influence of antagonists on effects of "homonymous" and "heteronymous" excitatory amino acids on both objects in order to estimate indirectly how different are the conformations (corresponding to the "moderately compressed" or "compressed" glutamate molecule) of structures determining the responses to excitatory amino acids which were investigated.

EXPERIMENTAL METHOD

The character of action of the test compounds on glutamate receptors was determined in physiological experiments on the tergocoxal muscle of the locust *Locusta migratoria migratorioides*. Diethyl esters of asparatic and glutamic acids (DEEAA and DEEGA respectively), 2-amino-3-phosphono-propionic acid (APPA), and 2-amino-4-phosphono-butanoic acid (APBA) were studied. Excitatory postsynaptic potentials (EPSP) and changes in membrane potential in response to microapplication of glutamate and aspartate were recorded with intracellular microelectrodes. EPSP were obtained by stimulation of the motor nerve with series of 3-10 stimuli, with a following frequency of 150 Hz. The nerve was stimulated by means of a suction electrode. To avoid the appearance of spikes and of contraction of the muscle, the physiological saline used contained a reduced concentration of Ca^{++}, and Mg^{++} ions were added to it. The blocking action of the antagonist was determined by measuring concentrations which reduced the amplitude of the EPSP, and also of the glutamate and aspartate responses by 50% (EC_{50}).

Seizure states were investigated on male albino mice weighing 18-25 g during the spring. Seizures were evoked by two methods: by injecting aspartate, glutamate, KU sulfate, and QA into the cerebral ventricles through the right lateral ventricle, and by subcutaneous injection of the standard convulsant metrazol. Aspartate and glutamate were obtained from Reanal (Hungary), AQ and KU from Sigma (USA). The dose of aspartate, glutamate, and KU was 50 μg,

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TABLE 1. Effect of Antagonists of Excitatory Amino Acids on Seizures Evoked by Injection of Aspartate, Glutamate, KU, and QA into Cerebral Ventricles of a Mouse

Antagonist (into cerebral ventricles)	Convulsant (into cerebral ventricles), μg			
	aspartate 50	glutamate 50	KU 50	QA 5
DEEAA	0 + 15 30	0 + 30 60	0 + 15 30—60	0 + 120 240
DEEGA	0 + 15 30	0 + 15 30	0 + 15 30—60	0 30—240
APPA	+ 5—25	0 5—25	0 + 5 12,5—25	0 5—25
APBA	0 + 5—12,5 25	0 + 5 12,5—25	+ 5—25	0 5—25

Legend. Numbers indicate doses of antagonists (in μg); +) prevention of seizures, 0) no effect.

TABLE 2. Concentrations of Antagonists of Glutamate Receptors Reducing Amplitudes of EPSP and Glutamate and Asparatate Responses of Locust Muscle Fibers

Antagonist	EC_{50}, M		
	EPSP	glutamate responses	aspartate responses
DEEAA	$7{,}9 \cdot 10^{-3}$	$1{,}5 \cdot 10^{-2}$	$5 \cdot 10^{-3}$
DEEGA	$2 \cdot 10^{-3}$	$3{,}2 \cdot 10^{-3}$	$3 \cdot 10^{-2}$
APPA	$1{,}2 \cdot 10^{-2}$	—	10^{-3}
APBA	$6 \cdot 10^{-4}$	$5 \cdot 10^{-4}$	$1{,}8 \cdot 10^{-3}$

that of QA 5 μg, and of metrazol 80 mg/kg body weight. The solutions were injected into the ventricles in a volume of 0.005 ml by means of a semiautomatic apparatus [6]. Antagonists of the excitatory aminoacids were injected into the cerebral ventricles or intraperitoneally, 3 and 30 min respectively before the convulsant. Control animals, which were present in each experiment, received injections of volumes of distilled water (intraperitoneally) or physiological saline (into the cerebral ventricles), with pH adjusted to that of the corresponding solution. The pH of solutions of asparatate, glutamate, KU, and QA was 5.0, 5.0, 2.5, and 5.0 respectively. All the anatgonists of the excitatory amino acids were tested in increasing doses, with approximately a twofold step. Four basic parameters of the seizures were determined: the latent period of onset of the seizures, number of animals with generalized clonic seizures, the number of animals with tonic extension, and mortality. Table 1 gives data only for the second parameter, for the remaining results did not differ significantly from the control [2].

EXPERIMENTAL RESULTS

Comparison of the structure of the test compounds with their ability to influence neuromuscular transmission in the locust showed that structural analogs of glutamic acid had a stronger blocking action than asparatate derivatives. DEEGA and APBA reduced the amplitude of EPSP in lower concentrations than DEEA and APPA. The esters studied blocked both glutamate and asparatate potentials in the muscle fibers. Glutamic acid esters were more active against glutamate potentials, aspartate esters were more active against aspartate potentials. However, no marked selectivity could be observed. All the esters were able to reduce the amplitude of both glutamate and aspartate potentials (Table 2). Concentrations reducing the amplitude of the glutamate and aspartate potentials differed. APPA reduced the amplitude of aspartate potentials only. Against EPSP, this compound had a much weaker action than APBA. Data in the literature on the ability of aspartate and glutamate to depolarize the muscle fiber membrane, and on the ability of esters of these amino acids to block neuromuscular transmission suggest that several populations of receptors, reacting

with different conformational forms of glutamic acid [1] are present in the locust muscle membrane. The stronger blocking activity of glutamic acid esters proved that glutamate receptors with a "slightly compressed" conformation play the most important role in the neuromuscular synapses of the locust. This hypothesis is in agreement with the results of the present investigation. KU and QA, in which the distance between the carboxyl groups corresponds to that in aspartic acid (i.e., the "compressed" conformation), even in a concentration of 2×10^{-2} M, did not affect the membrane potential of the locust muscle fibers.

Seizures evoked by injection of aspartate and glutamate into the cerebral ventricles were depressed most effectively by the corresponding antagonists (Table 1). It was found, however, that the antagonists could also inhibit seizures evoked by "heteronymous" convulsants. For example, DEEGA, in a dose of 30 μg, prevented seizures induced both by glutamate and by aspartate. Some differences were observed in the effective doses during the action of APBA, which prevented the development of seizures in response to glutamate is a smaller dose than in response to aspartate. Kynurenin seizures were prevented much more easily by the test antagonists than seizures induced by QA (Table 1). The latter were inhibited by only one antagonist, namely DEEAA, and only in a dose of 240 μg, which exceeded its effective doses against other excitatory amino acids by 4-8 times. The tested antogonists prevented seizures induced by KU and aspartate in about equal doses. DEEGA and DEEAA is a dose of 30 μg prevented both types of seizures but did not affect seizures induced by glutamate. ATPA was more effective against aspartate than against KU, whereas APBA, on the contrary, was more effective against KU.

DEEGA and DEEAA in doses of up to 400 mg/kg, and APPA and APBA in doses of up to 50-100 mg/kg, when injected intramuscularly, did not affect seizures evoked by excitatory amino acids or by metrazol in any single case. The low effectiveness of the antagonists when injected by this method can probably be explained by their poor penetration through the blood-brain barrier.

Investigation of the effect of antagonists on the development of seizures showed that they do not possess narrow selectivity against one particular convulsant belonging to the excitatory amino acids. Antagonists structurally closer to aspartic acid prevented seizures in response to aspartate and KU in lower doses than glutamate derivatives. This observation suggests that glutamate receptors of neurons of brain structures in the wall of the ventricles are closer in the spatial configuration of their active centers to glutamate receptors, corresponding to the "compressed" conformational form of glutamic acid. The ability of DEEAA, DEEGA, APPA, and APBA to block the seizure effects of KU and their ineffectiveness against QA suggest that these two endogenous convulsants act on different brain structures. Inactivity of KU and QA on locust fibers can probably be explained by differences in glutamate reception in this species of animal.

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