In this paper we have in fact dealt with only the last stage of delayed adaptation of the animal to hyperbaric oxygen. It will be perfectly evident that to understand the mechanism of onset of oxygen epilepsy, it is important to have some idea of changes in monoamine metabolism in the preconvulsion stage, characterized on the basis of the EEG by alternation of three states: transitional, compensated, and preconvulsive [8].

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SELECTIVE ANTICONVULSANT ACTION OF N-SUBSTITUTED IMIDAZOLE-4,5-DICARBOXYLIC ACIDS AGAINST QUINOLINIC ACID

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A new trend in research into convulsive states in recent years has been the study of neuroactivity of tryptophan metabolites — kynurenine (KYN) and quinolinic acid (QUA) — as endogenous convulsants [7]. Their convulsant effect, established initially in mice, sexually immature rats, and frogs [7], has also been observed in experiments on rats when injected into the cerebral ventricles (KYN) [12] and into the dorsal hippocampus (QUA) [13]. Most information about the antagonists of the convulsant action of KYN and QUA has been obtained in mice. Four groups of antagonists were distinguished in experiments on these animals: 1) KYN metabolites (kynurenins) — kynurenic, picolinic, xanthurenic, and nicotinic acids; 2) inhibitory amino acids — taurine and glycine; 3) GABA derivatives — fenibut, baclofen, and sodium hydroxybutyrate; 4) standard anticonvulsants — phenobarbital, phenytoin sodium, and methindione. However, virtually all these antagonists were effective only against KYN and not against QUA. The only antagonists of QUA, namely fenibut, baclofen, and sodium hydroxybutyrate, proved to be ineffective in experiments on rats [3]. Because of this state of affairs, the search for antagonists predominantly of QUA is an urgent problem. The investigation described below was undertaken to solve this problem.

N-substituted derivatives of imidazole-4,5-dicarboxylic acid, which can be regarded as QUA analogs (Table 1), were chosen for testing. It was shown previously [8] that to counter-

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TABLE 1. Chemical Structure of N-Substituted Imidazole-4,5-Dicarboxylic Acids and of Excitatory Amino Acids

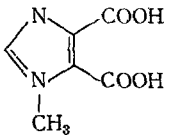
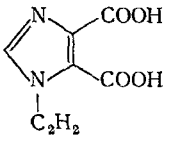
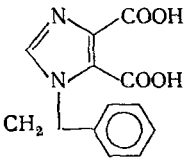
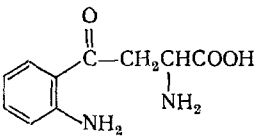
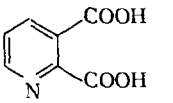
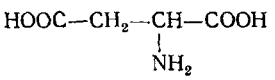
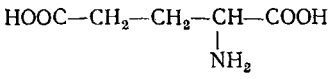
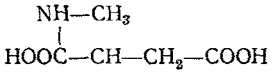
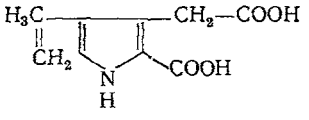
Compound	Name	Structural formula	Molecular weight, daltons
I	1-Methylimidazole-4,5-dicarboxylic acid		170,12
II	1-Ethylimidazole-4,5-dicarboxylic acid		184,15
III	1-Benzylimidazole-4,5-dicarboxylic acid		246,22
IV	L-Kynurenin sulfate		306,1
V	QUA		167,3
VI	Aspartic acid		133,1
VII	Glutamic acid		147,1
VIII	N-Methyl-DL-aspartic acid		147,1
IX	Kainic acid		213,0

TABLE 2. Convulsant and Anticonvulsant Action of N-Substituted Imidazole-4,5-Dicarboxylic Acids against Excitatory Amino Acids

Compound	Intrinsic convulsant effect, dose in μg	Anticonvulsant effect: convulsant and its dose (in μg) injected into cerebral ventricle						preparations given intraperitoneally, mg/mg, QUA,
		KYN (50)	QUA (5)	ASP (50)	GLU (25)	MASP (0.25)	KAI (0.1)	
I (IEM 1573)	0 C 5 25	0 10	0 + 2,5 5	0 10	0 10	0 + 2,5 5	0 10	0 200—600
II (IEM-1442)	0 C 5—25 50	0 + 2,5 5	0 + 1 2,5	0 + 2,5 5	0 + 10 25	0 + 0,5 2,5	0 + 2,5 5	0 + 100 200
III (IEM-1441)	0 C 5—25 50	0 + 5 10	0 + 5 10	0 25	0 + 10 25	0 + 10 25	0 25	0 + 100 200

act the convulsant effect of KYN and QUA, dicarboxylic metabolites of tryptophan, it is necessary to "cover" at least two active groups: amino and carboxyl. To assess the degree of selectivity of anticonvulsant action of the imidazole derivatives, other excitatory amino acids were also used in addition to KYN and QUA: aspartic (ASP) and glutamic (GLU) acids and their synthetic analogs: N-methyl-DL-aspartic and kainic acids (MASP and KAI).

EXPERIMENTAL METHOD

Experiments were carried out on male albino SHR mice weighing 14-25 g, from the Rappolovo Nursery, Leningrad Region, in the winter and spring period. During the experiment the mice were kept in metal boxes measuring 20 × 15 × 10 cm (six mice in each group). The experiments were carried out usually between 10 a.m. and 4 p.m.

The preparations for testing — 1-methyl-, 1-ethyl-, and 1-benzylimidazole-4,5-dicarboxylic acids (compounds I, II, and III respectively) were synthesized by methods described previously [1, 5]. For injection into the cerebral ventricles compound II was dissolved in distilled water with heating to 50°C (the pH of the test solutions was 3.0-4.0). Compounds I and III were dissolved in distilled water with heating to 70-80°C, and 10N NaOH was added (the pH of the solutions of both preparations was 7.0-8.0). For intraperitoneal injections, solutions of the preparations were made up in the emulsifier Tween-85.

The following excitatory amino acids were used in convulsive (ED_{100}) doses: KYN as the sulfate L-KYN (from Sigma, USA) 50 μg, QUA (Sigma) 5 μg; ASP (Reanal, Hungary) 50 μg, GLU (USSR) 25 μg, MASP (Sigma) 0.25 μg, KAI (generously provided by Dr. A. M. Zharkovskii) 0.1 μg. All the excitatory amino acids were dissolved in distilled water, and then heated to 50-60°C (for KYN and QUA), 100°C (for ASP and GLU), and 30-40°C (for MASP and KAI).

The test compounds were injected into the cerebral ventricles of a conscious mouse by means of a semiautomatic apparatus, by the method in [6]. After 3 min, the excitatory amino acids in convulsant doses were injected similarly into the cerebral ventricles. All solutions were injected into the cerebral ventricles in a volume of 0.005 ml. The time of one injection was 1 sec. Intraperitoneal injections were given 30 min before injection of the convulsant into the cerebral ventricles. Four principal parameters of convulsions were determined: 1) the latent period of onset of clonic convulsions; 2) the number of animals with clonic convulsions (the frequency of clonic convulsions in the group); 3) the number of animals with tonic extensions (the frequency of tonic extensions in the group); 4) mortality. Since differences between the groups were exhibited most clearly with respect to the frequency of clonic convulsions in the group, this parameter is illustrated in Table 2. The significance of differences was estimated from Genes' tables [2]. In each experiment a control group of animals was used for each convulsant, and before the convulsant, physiological saline was injected into their cerebral ventricles.

EXPERIMENTAL RESULTS

A study of the effectiveness of injection of compounds I, II, and III into the cerebral ventricles showed that, in certain doses, they themselves cause excitation and convulsions. For instance, when compounds II and III were injected, convulsions began to appear with a dose of 50 μg, whereas when compound I was injected, they began to appear with dose of 25 μg. In the subsequent tests, therefore, smaller doses of these substances were used.

It will be clear from Table 2 that all three preparations exhibited selective antagonism toward convulsions induced by QUA and MASP. This selectivity was most marked in the case of compounds I and II, and less marked with compound III, which prevented convulsions induced not only by QUA, but also by KYN, the precursor of QUA, equally effectively. Of the preparations studied, the widest spectrum of antagonism was possessed by compound II, which prevented convulsions caused by all the excitatory amino acids (Table 2). Compounds II and III preserved their anticonvulsant effect against QUA even when injected systemically (intraperitoneally).

Comparison of the structural formulas of the compounds shows that selectivity for MASP and its structurally rigid cyclic analog QUA was more marked in the case of preparations with a comparatively "lighter" radical attached to the nitrogen atom of the imidazole ring. Compound III, with a "heavy" radical at this nitrogen atom, was less selective, although it prevented convulsions induced by QUA even when injected systemically, probably due to the greater lipophilicity of the molecule and its better ability to pass through the blood–brain barrier. The N-substituted derivatives of imidazole-4,5-dicarboxylic acid studied have a fragment of their chemical structure including the two carboxyl groups attached to neighboring carbon atoms and a nitrogen atom in the α-position (Table 1), which determines the functional and receptor activity of these substances, common with QUA and MASP [7, 8]. It is the presence of this common functionally significant fragment of their structure that most probably determines the anticonvulsant antagonism of this class of preparations with QUA and MASP, which is evi-

dently competitive in type. This also suggests that QUA and MASP react with the common (N-methyl-D-aspartate) receptor.

It was shown previously that the excitatory effect of QUA on rat cerebral cortical neurons is reduced by phosphonic acid derivatives (2-amino-5-phosphonovalerianic and 2-amino-7-phosphonoheptanic acids) — predominantly blockers of MASP-receptors [10, 11]. Selectivity of the anticonvulsant action of the imidazole derivatives studied against QUA supports the hypothesis put forward previously [10, 14] that QUA acts through MASP-receptors.

Thus preparations with a selective anticonvulsant action against an endogenous convulsant (QUA) were found for the first time. It will be useful to continue this investigation on rats, for species differences are known to exist in the effectiveness of KYN and QUA antagonists. Besides the differences in the activity of the GABA derivatives mentioned above, it has also been found that kynurenic acid is ineffective against QUA in experiments on mice [9], but it is very effective in experiments on rats [3, 4]. A similar difference has also been established for glycine [3, 9].

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