Combined Blockade of α3β4 Nicotinic Acetylcholine Receptors and GluR1 AMPA Receptors in Rats Prevents Kainate-Induced Tonic-Clonic Seizures

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Intramuscular injection of IEM-1754, a blocker of cerebral GluR1 AMPA receptors, in doses of 0.5-3.0 mg/kg decreased the incidence of kainate-induced tonic-clonic seizures and mortality rate by 2.7-4 times. IEM-1678, an α3β4 nicotinic acetylcholine receptor antagonist administered intramuscularly in a maximum dose of 3 mg/kg decreased the incidence of kainate-induced tonic-clonic seizures and mortality rate by 2.3-2.7. IEM-1460 blocking both GluR1 AMPA receptors and α3β4 nicotinic acetylcholine receptors, injected intramuscularly in doses of 0.5-3.0 mg/kg produced the maximum anticonvulsant activity and 8-fold decreased the incidence of kainate-induced tonic-clonic seizures and mortality rate.

Key Words: *IEM-1460 [1-trimethylammonio-5-(1-adamantane-methyl-ammonio)-pentane dibromide]; IEM-1678 (tertiary-butyldecylammonium chloride); IEM-1754 [1-amino-5-(1-adamantylmethylamino)-pentane dihydrobromide]; kainate; seizures*

Blockade of GluR1 AMPA receptors abolishes the toxic effects of kainate and glutamate inducing degeneration of hippocampal neurons [9,11]. This is why AMPA antagonists completely prevent kainate-induced local clonic seizures resulting from kainate-induced degeneration of GABAergic interneurons [6,11,13], but only partially prevent kainate-induced generalized tonic-clonic seizures, because they are unable to decrease massive release of endogenous glutamate stimulating NMDA receptors on pyramidal neurons [6,12,13].

Nicotine significantly potentiates glutamate release in hippocampal interneurons and pyramidal neurons due to stimulation of presynaptic α3β4 nicotinic acetylcholine receptors [3]. Mecamylamine, an antagonist of brain α3β4 nicotinic acetylcholine receptors and NMDA receptors, prevents epileptogenesis of tonic-clonic seizures induced by nicotine and maximum electric shock through decreasing the release of endogenous glutamate and blockade of NMDA receptors on pyramidal neurons [3,4, 8,10]. However, mecamylamine only slightly decreases the severity of generalized kindling limbic seizures [10] caused by activation of GluR1 AMPA receptors on interneurons by endogenous glutamate [5].

The agents blocking simultaneously GluR1 AMPA receptors and α3β4 nicotinic acetylcholine receptors attract much attention in this respect, because these drugs should be more potent than antagonists of one type of these receptors in preventing tonic-clonic seizures induced by kainate in toxic doses. Experiments on hippocampal slices showed that IEM-1754 and IEM-1460 block GluR1 AMPA receptors on interneurons [2,14]. IEM-1460 and IEM-1678 are antagonists of nicotinic acetylcholine receptors in parasympathetic ganglia [1, 15]. These ganglia mainly include nicotinic acetylcholine receptors of the α3β4 type [7].

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Here we compared the incidence of kainate-induced tonic-clonic seizures and mortality rate after treatment with IEM-1754 (GluR1 AMPA receptor antagonist), IEM-1678 (α3β4 nicotinic acetylcholine receptor antagonist), and IEM-1460 (antagonist of GluR1 AMPA receptors and α3β4 nicotinic acetylcholine receptors).

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 180-200 g. The study was conducted at 10.00-16.00. Tonic-clonic seizures in rats were induced by intramuscular injection of kainate in a toxic dose of 12 mg/kg. The rats received intramuscular injections of the test compounds (IEM-1754, IEM-1678, and IEM-1460; Institute of Experimental Medicine) 30 min before kainate administration. The incidence of tonic-clonic seizures (percents of the total number of animals) and mortality rate were recorded in each group over 180 min after kainate treatment.

The results were analyzed by Student's test.

RESULTS

Kainate in a toxic dose of 12 mg/kg induced typical behavior associated with activation of limbic structures (locomotor and exploratory hyperactivity, stereotypic behavior, and wet dog shakes) 15-20 min after intramuscular injection. Local clonic seizures developed in 100% rats (duration 30-60 min) 50-70 min after kainate administration and were followed by generalized tonic-clonic seizures in 80% animals eventuating in death of 40% rats over 180 min postinjection (Table 1).

IEM-1678, a selective blocker of parasympathetic ganglia [15] containing primarily α3β4 nicotinic acetylcholine receptors [7], in low doses of 0.1 and 0.3 mg/kg had no effect on the severity of kainate-induced tonic-clonic seizures (Table 1), while in a dose of 1 mg/kg it decreased the severity of tonic-clonic seizures and mortality rate by 1.5 and 1.3 times, respectively (insignificant changes). Intramuscular injection of IEM-1678 in the maximum dose of 3 mg/kg reduced the incidence of tonic-clonic seizures and mortality rate by 2.3 and 2.7 times, respectively ($p<0.05$, Table 1).

Nonselective blockade of cerebral α3β4 nicotinic acetylcholine receptors induced by systemic administration of mecamylamine only slightly decreases the severity of tonic-clonic kindling seizures [10] caused by activation of GluR1 AMPA receptors on interneurons with endogenous glutamate [5].

Our experiments showed that systemic administration of IEM-1678, a selective α3β4 nicotinic acetylcholine receptor antagonist, produces minor inhibitory effect on kainate-induced tonic-clonic seizures. These data suggest that blockade of central α3β4 nicotinic acetylcholine receptors has little effect on generation of kainate-induced seizures resulting from activation of GluR1 AMPA receptors on interneurons with kainate and endogenous glutamate [6,11-13].

Intramuscular injection of IEM-1754 in a dose of 0.1 mg/kg slightly decreased the severity of kainate-induced seizures, while in doses of 0.5-3.0 mg/kg decreased their incidence and mortality rate by 2.7-4 times ($p<0.05$, Table 1). Hence, IEM-1754, a blocker of GluR1 AMPA receptors on interneurons [2] is more potent that α3β4 nicotinic acetylcholine receptor antagonist IEM-1678 in decreasing the severity of tonic-clonic seizures.

Nonselective AMPA receptor blockers completely prevent kainate-induced local clonic seizures [6,11,13]. However, these drugs only partially prevent generalized tonic-clonic seizures, because they are unable to decrease the release of endogenous glutamate stimulating NMDA receptors on pyramidal neurons [6,12,13]. Similarly to nonselective AMPA receptor antagonists, IEM-1754 partially prevented the development of kainate-induced generalized tonic-clonic seizures in rats (Table 1). Therefore, selective blockade of GluR1 AMPA receptors is not sufficient for preventing epileptogenesis of kainate-induced tonic-clonic sei-

TABLE 1. Effect of Compounds on the Severity of Seizures Induced by Systemic Administration of Kainate in a Dose of 12 mg/kg

Compound (mg/kg)+kainate (12 mg/kg)	Seizures, %	Mortality rate, %
Control, kainate (12 mg/kg)	80	40
IEM-1460, 0.03	60	30
IEM-1460, 0.1	30*	15*
IEM-1460, 0.5	10***	5***
IEM-1460, 3.0	10***	5***
IEM-1754, 0.1	60	30
IEM-1754, 0.5	30*	15*
IEM-1754, 3.0	20**	10**
IEM-1678, 0.1	80	40
IEM-1678, 0.3	70	35
IEM-1678, 1.0	55	30
IEM-1678, 3.0	35*	15*

Note. *$p<0.05$, **$p<0.02$ and ***$p<0.01$ compared to the control.

zures, because cannot prevent their generalization by endogenous glutamate in pyramidal neurons.

IEM-1460, a blocker of both GluR1 AMPA receptors and α3β4 nicotinic acetylcholine receptors [1,14], in a dose of 0.03 mg/kg slightly decreased the severity of kainate-induced seizures, while in a dose of 0.1 mg/kg 2.7-fold decreased the severity of kainate-induced tonic-clonic seizures and mortality rate ($p<0.05$, Table 1). In a dose range of 0.5-3.0 mg/kg IEM-1460 almost completely prevented kainate-induced tonic-clonic seizures (decreased the incidence and mortality rate from seizures by 8 times, $p<0.02$; Table 1).

Nonselective α3β4 nicotinic acetylcholine receptor antagonist mecamylamine decreases the release of endogenous glutamate in pyramidal neurons and prevents the development of generalized tonic-clonic seizures induced by toxic doses of nicotine and maximum electric shock [3,4,8,10]. The more complete suppression of tonic-clonic seizures by IEM-1460 compared to selective cerebral GluR1 AMPA receptor antagonist IEM-1754 can be explained by additional blockade of presynaptic α3β4 nicotinic acetylcholine receptors on pyramidal neurons significantly reducing the release of endogenous glutamate, which is required for the generalization of kainate-induced seizures in pyramidal cells [8].

We conclude that combined blockade of GluR1 AMPA receptors and α3β4 nicotinic acetylcholine receptors in the brain provides maximum protection from epileptogenesis of kainate-induced generalized tonic-clonic seizures.

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