Peripheral and Central Routes of Administration of Quaternary Ammonium Compound IEM-1460 are Equally Potent in Reducing the Severity of Nicotine-Induced Seizures in Mice

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Peripheral administration of nicotinic receptor antagonists with a quaternary ammonium group (hexamethonium and chlorisondamine) did not prevent the development of seizures induced by systemic treatment with nicotine in the toxic dose. The Me_3N^+ group with stable positive charge inhibits transport of these compounds into the brain through the blood-brain barrier. Intracerebral and peripheral (intraperitoneal) administration of compound IEM-1460 with the Me_3N^+ group was equally potent in reducing the severity of nicotine-induced seizures in mice. This phenomenon is related to the fact that IEM-1460 acts as a nicotinic receptor antagonist and polyamine agonist, which increases blood-brain barrier permeability for polar compounds. These features contribute to IEM-1460 transport into the brain. High anticonvulsant activity of IEM-1460 on the model of nicotine-induced seizures is associated with combined blockade of nicotinic receptors ($\alpha3\beta4$ receptors) and glutamate receptors (GluR1 AMPA receptors).

Key Words: *IEM-1460; arcaine; nicotine; seizures; blood-brain barrier*

Published data show that intracerebral administration of nicotinic receptor antagonists carrying a quaternary ammonium group (hexamethonium and chlorisondamine) prevents the development of seizures in mice and rats induced by peripheral treatment with nicotine in a toxic dose [3]. Peripheral administration of nicotinic receptor antagonists with the Me_3N^+ group has no effect on the severity of nicotine-induced seizures. The Me_3N^+ group with stable positive charge inhibits transport of these compounds into the brain through the blood-brain barrier (BBB). Compound IEM-1460 contains Me_3N^+ group and *in vivo* blocks nicotinic receptors in parasympathetic ganglia (primarily $\alpha3\beta4$ nicotinic receptors) and AMPA glutamate receptors on hippocampal interneurons (Ca^{2+}-permeable AMPA receptors with no GluR2 subunit, GluR1 AMPA receptors) [5].

In vivo experiments showed that peripheral administration of IEM-1460 prevents the development of seizures in rats induced by systemic treatment with nicotine or kainate in the toxic dose [2,4]. It remains unclear why the compound carrying quaternary Me_3N^+ group and not crossing through BBB is capable of preventing the development of central seizures.

Our work was designed to study this phenomenon. We compared the anticonvulsant effects of peripheral and central treatment with IEM-1460 in mice with nicotine-induced seizures.

MATERIALS AND METHODS

Experiments were performed on 134 male albino mice weighing 18-20 g. The mice were maintained

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in a vivarium and had free access to food and water. The study was conducted at 10.00-16.00. Nicotine in a convulsive dose was injected intraperitoneally (20 mg/kg, systemic treatment) or intracerebroventricularly (i.c.v., 15 μg in 5 μl per mouse, central treatment) to mice of the control and treatment groups (IEM-1460 and arcaine). Injection into lateral ventricles of the brain was performed using a stereotaxic injector [6]. The experiment was performed in 9 series. Each group consisted of 10-34 mice (Table 1).

Each series was started from administration of nicotine in a convulsive dose to control animals. When constant seizures occurred in control animals, seizure activity was studied in mice of the treatment group after combined administration of test substances and nicotine.

The central and peripheral anticonvulsant effects of IEM-1460 were evaluated in several series. In series I, systemic treatment with nicotine in the convulsive dose (20 mg/kg intraperitoneally) was performed 20 min after systemic administration of IEM-1460 (1 and 10 mg/kg). In series II, central injection of IEM-1460 (0.5 and 5 μg i.c.v.) was followed by systemic treatment with nicotine (20 mg/kg intraperitoneally, after 5 min). In a special series, systemic administration of IEM-1460 (1 mg/kg intraperitoneally) was performed 20 min before central injection of nicotine in a convulsive dose (15 μg i.c.v.).

Nicotine (20 mg/kg intraperitoneally) was injected 20 min after combined administration of the polyamine antagonist arcaine (10 mg/kg intraperitoneally) and IEM-1460 (1 mg/kg intraperitoneally) to evaluate the contribution of the polyamine site in NMDA receptors to anticonvulsant activity of IEM-1460. The combined effect of IEM-1460 and arcaine on nicotine-induced seizures was compared with individual anticonvulsant action of arcaine and IEM-1460. These substances were injected intraperitoneally 20 min before administration of nicotine in a convulsive dose.

The incidence and mortality rate from tonic-clonic seizures were recorded in each group over 30 min after nicotine administration (percent of total number of animals). After i.c.v. injection of nicotine and IEM-1460, the animals were killed by ether anesthesia. The accuracy of i.c.v. injection was verified. The animas were scalped. A needle was inserted perpendicularly into the hole in the skull. The depth of needle insertion was similar to that in experiments with the test substances. Methylene blue (5 μl) was infused through the needle.

Compound IEM-1460 ($[Me_3N^+\text{-}(CH_2)_5\text{-}N^+H_2CH_2Ad] \times 2Br^-$; Ad, 1-adamantyl $C_{10}H_{15}$ radical) was synthesized at the Institute of Experimental Medicine. This compound is listed in the Tocris catalogue (IEM-1460). Other reagents were manufactured by Sigma.

The results were analyzed by Fischer's exact test.

RESULTS

Published data show that IEM-1460 *in vitro* blocks GluR1 AMPA receptors on hippocampal interneurons, as well as nicotinic receptors in sympathetic and parasympathetic ganglia ($\alpha3\beta4$ receptors) [5]. Systemic intraperitoneal injection of IEM-1460 was 2-3-fold more potent than selective $\alpha3\beta4$ nicotinic receptor antagonist IEM-1678 in preventing the development of tonic-clonic seizures in rats induced by intramuscular administration of nicotine in a toxic dose. IEM-1678 contains a tertiary amino group and, therefore, easily crosses BBB. It was hypothesized that higher anticonvulsant activity of IEM-1460 *vs*. IEM-1678 on the model of seizures induced by peripheral administration of nicotine is related to combined blockade of presynaptic $\alpha3\beta4$ nicotinic receptors and postsynaptic GluR1 AMPA receptors on hippocampal interneurons and pyramidal cells [4].

It remains unclear whether peripheral administration of IEM-1460 can produce a direct central effect. Peripheral administration of quaternary nicotinic receptor antagonists (hexamethonium and chlorisondamine) has no effect on the severity of nicotine-induced seizures, since positively charged Me_3N^+ group inhibits transport of these compounds into the brain through BBB.

Injection of nicotine in a dose of 20 mg/kg caused tonic-clonic seizures in 100% mice. Table 1 shows that 62% animals die under these conditions. Intracerebroventricular administration of nicotine in a dose of 15 μg caused tonic-clonic seizures in 90% mice. The mortality rate of animals was 10%.

Pretreatment with IEM-1460 in a dose of 1 mg/kg had little effect on the incidence of seizures, but significantly decreased the mortality rate of mice with nicotine-induced seizures (by 12 times, $p<0.01$). IEM-1460 in a dose of 10 mg/kg 2-fold decreased the incidence of seizures and prevented the death of animals from nicotine-induced seizures ($p<0.01$).

Preliminary i.c.v. injection of IEM-1460 in a dose of 0.5 μg decreased the mortality rate and incidence of nicotine-induced seizures in mice (by 2.2 and 1.4 times, respectively, $p<0.05$). IEM-1460 in a dose of 5 μg completely prevented death of mice and 2-fold decreased the incidence of nicotine-induced seizures ($p<0.01$, Table 1).

TABLE 1. Effects of IEM-1460 and Arcaine on the Incidence and Mortality Rate from Nicotine-Induced Seizures

Preparation	*n*	Seizures, %	Mortality rate, %
Nicotine (control)*	34	100	61.8
Nicotine (control)**	20	90	10
IEM-1460 (1 mg/kg) and nicotine*	20	85	5^{+}
IEM-1460 (1 mg/kg) and nicotine**	10	50^{o}	0
IEM-1460 (10 mg/kg) and nicotine*	10	50^{+}	0^{+}
IEM-1460 (0.5 μg)*** and nicotine*	10	70^{o}	30
IEM-1460 (5 μg)*** and nicotine*	10	50^{+}	0^{+}
Arcaine (10 mg/kg) and nicotine*	10	90	40
Arcaine (10 mg/kg), IEM-1460 (1 mg/kg), and nicotine*	10	100	30

Note. *Intraperitoneal injection, 20 mg/kg; **i.c.v. injection, 15 μg; ***administration of nicotine 5 min after i.c.v. injection (20-min interval between drug treatment in other experiments). $^{+}p<0.01$ and $^{o}p<0.05$ compared to the control.

Experiments on mice with nicotine-induced tonic-clonic seizures showed that intraperitoneal and intracerebral injections of IEM-1460 produced similar anticonvulsant effect. These data indicate that the anticonvulsant effect of IEM-1460 in different routes of treatment is mediated by the same central mechanism.

Preliminary intraperitoneal injection of IEM-1460 in a dose of 1 mg/kg significantly decreased the incidence of seizures induced by i.c.v. administration of nicotine (by 1.8 times, $p<0.05$, Table 1). Hence, systemic administration of IEM-1460 produced an anticonvulsant effect due to transport into the brain through BBB. It should be emphasized that IEM-1460 contains the Me_3N^+ group with stable positive charge, which prevents transport through BBB. Hence, the pathway of IEM-1460 transport into the brain remains unclear.

Our previous studies showed that IEM-1460 has properties of a GluR1 AMPA receptor antagonist and NMDA receptor polyamine site agonist [1]. Systemic intraperitoneal administration of polyamine agonist IEM-1460 potentiates the development of seizures induced by intraperitoneal injection of NMDA. By contrast, administration of polyamine antagonist arcaine prevents seizures induced by intraperitoneal injection of NMDA. The observed effects are associated with opposite changes in BBB permeability to NMDA, which results from modulation of the NMDA receptor polyamine site in BBB cells [1].

Intraperitoneal injection of arcaine in a dose of 10 mg/kg slightly decreased the incidence and mortality rate from seizures induced by intraperitoneal nicotine (Table 1). These data indicate that the decrease in BBB permeability to the polar compound induced by a polyamine antagonist arcaine [1] was not followed by significant reduction in BBB permeability to lipophilic nicotine. As differentiated from polar compound NMDA, nicotine easily crosses BBB.

Previous experiments on mice with NMDA-induced seizures showed that combined intraperitoneal administration of IEM-1460 and arcaine abolishes the anticonvulsant effects of IEM-1460 and arcaine due to the opposite action on BBB permeability to NMDA. Competitive antagonism exists between the test substances at the NMDA receptor polyamine site in BBB cells [1].

Combined intraperitoneal injection of 10 mg/kg arcaine and 1 mg/kg IEM-1460 was followed by a significant decrease in the anticonvulsant effect of IEM-1460 on mice with nicotine-induced seizures (Table 1). Arcaine and IEM-1460 probably has little effect in BBB permeability to highly lipophilic nicotine. Hence, the observed effect is probably associated with a decrease in IEM-1460 concentration in the brain due to inhibition of drug transport through BBB. These changes are related to arcaine-induced blockade of the NMDA receptor polyamine site in BBB cells.

Intraperitoneally injection of polyamine agonist IEM-1460 is probably followed by drug transport in the brain, which results from opening of BBB to polar compounds. IEM-1460 has a spermine-like activating effect on the NMDA receptor polyamine site in BBB cells.

Our results explain the unexpectedly strong anticonvulsant effect of systemic treatment with test substance, which belongs to the class of quaternary ammonium compounds. These compounds are usually ineffective or low effective in experimental seizures. Systemic administration of a quaternary compound IEM-1460 has high anticonvulsant activity in mice with nicotine-induced seizures. The observed changes are associated not only with blockade of $\alpha 3\beta 4$ nicotinic receptors and GluR1 AMPA receptors in the brain, but also with spermine-like effect of IEM-1460. This property provides trans-

port of IEM-1460 into the brain due to the increase in BBB permeability for polar compounds.

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