Combined Blockade of AMPA and NMDA Receptors in the Brain of Rats Prevents Pentylenetetrazole-Induced Clonic and Tonic-Clonic Seizures without Ataxia

V. E. Gmiro and S. E. Serdyuk

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 6, pp. 675-677, June, 2008
Original article submitted May 22, 2007

Intramuscular injection of selective NMDA receptor antagonists memantine and arcaine 4-fold decreased the incidence of pentylenetetrazole-induced generalized tonic-clonic seizures in rats, while the incidence of clonic seizures decreased by 1.2-1.3 times. Memantine and arcaine are characterized by low therapeutic index, *i.e.* induced ataxia in rats in doses exceeding the effective anticonvulsant dose by only 3.5-10 times. Intramuscular injection of IEM-1913 (combined blockade of NMDA and AMPA receptors in the brain) decreased the incidence of pentylenetetrazole-induced clonic and tonic-clonic seizures in rats by 4-8 times. The therapeutic index of IEM-1913 surpassed that of memantine and arcaine by 200-600 times.

Key Words: *IEM-1913; memantine; arcaine; pentylenetetrazole; seizures*

Pentylenetetrazole in toxic doses induces massive release of endogenous glutamate in various structures of the brain [4,5]. Pentylenetetrazole-induced seizures in rats are associated with glutamate activation of AMPA and NMDA receptors in the brain [5,6,14]. NMDA receptor blockade with MK-801, memantine, and arcaine significantly decreases the severity of generalized tonic-clonic seizures [2,3, 14], but has no effect on pentylenetetrazole-induced local clonic seizures in rats [12,14]. Another disadvantage of NMDA receptor antagonists is low therapeutic index. Ataxia and stereotypy in rats are observed after treatment with these drugs in doses, which exceed the effective anticonvulsant dose only by several times [2,3,13].

Combined administration of NMDA and AMPA receptor antagonists in low doses prevents not only tonic-clonic seizures, but also kindled clonic seizures. This treatment causes no side effects, which are usually observed after separate administration of NMDA and AMPA receptor antagonists in high doses [7,9,11]. We hypothesized that combined blockade of NMDA and AMPA receptors in the brain can prevent clonic and tonic-clonic seizures. Published data show that epileptogenesis of kindled and pentylenetetrazole-induced seizures is associated with stimulation of NMDA and AMPA receptors with endogenous glutamate [6,7,14].

Our previous studies showed that compound IEM-1913 causes combined blockade of NMDA and AMPA receptors in the brain and is more potent than NMDA receptor antagonist memantine in reducing the severity and duration of allergic encephalomyelitis in rats [1].

Here we compared the anticonvulsant effect and safety (therapeutic index) of IEM-1913 and selective NMDA receptor antagonists memantine and arcaine.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 180-200 g. The animals were

Department of Neuropharmacology, Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg, Russia. ***Address for correspondence:*** g2119@online.ru. V. E. Gmiro

0007-4888/08/1456-0728

maintained in a vivarium and had free access to food and water. Pentylenetetrazole in a toxic dose of 70 mg/kg was injected intramuscularly to induce seizures [2]. Local clonic seizures (forelimb or hindlimb clonus) and generalized tonic-clonic seizures in rats (clonic seizures and tonic hindlimb extension) were recorded over 30 min after pentylenetetrazole injection.

Distilled water (control), mixed NMDA/AMPA receptor antagonist IEM-1913 (study drug), and selective NMDA receptor antagonists memantine and arcaine (reference drugs) were injected intramuscularly 30 min before pentylenetetrazole injection.

Compound IEM-1913 (N-(4-aminobutyl)-1-aminoadamantane dihydrobromide) was synthesized at the Institute of Experimental Medicine (Russian Academy of Medical Sciences). Pentylenetetrazole, arcaine, and memantine were manufactured by Sigma.

The incidence and mortality from pentylenetetrazole-induced clonic and tonic-clonic seizures were recorded in each group (percent of the total number of animals). We studied the capacity of IEM-1913, memantine, and arcaine to decrease the incidence of pentylenetetrazole-induced clonic and tonic-clonic seizures. Toxicity of study drugs was estimated from the ataxia-inducing dose (uncoordinated movements, fall on the side, and impairment of antigravitational responses) in 50% rats (TD_{50}). Drug safety was evaluated from the therapeutic index. This index was calculated as the ratio of the toxic dose inducing ataxia in 50% rats (TD_{50}) to the dose preventing pentylenetetrazole-induced tonic-clonic seizures in 50% rats (ED_{50}) [10,13].

The control and treatment groups included 8-10 animals. The results were analyzed by Fischer test.

RESULTS

Local clonic seizures (forelimb or hindlimb clonus) in 100% rats were observed 3-5 min after systemic administration of pentylenetetrazole in a toxic dose of 70 mg/kg. Generalized tonic-clonic seizures with tonic hindlimb extension were revealed in 80% rats 10-12 min after pentylenetetrazole injection. The seizure lasted for 5-15 min and resulted in death of 5% animals (Table 1).

Arcaine (1 mg/kg) and memantine (5 mg/kg) did not prevent the development of pentylenetetrazole-induced clonic seizures, but slightly decreased the incidence of tonic-clonic seizures (by 1.15-1.3 times, $p<0.2$; Table 1). Arcaine and memantine in the maximum dose (5 and 20 mg/kg, respectively) decreased the incidence of tonic-clonic seizures by 4 times ($p<0.05$, Table 1). These drugs had little effect on the incidence of clonic seizures (1.17-1.3-fold decrease, $p<0.2$; Table 1). Our results are consistent with published data that selective NMDA receptor antagonists significantly decrease the incidence of pentylenetetrazole-induced generalized tonic-clonic seizures, but do not prevent the clonic phase of pentylenetetrazole-induced seizures [12, 13,14].

Combined blockade of NMDA and AMPA receptors in the brain was induced by IEM-1913 [1]. IEM-1913 in the minimum dose of 0.01 mg/kg decreased the incidence of pentylenetetrazole-induced clonic and tonic-clonic seizures by 1.5-1.8 times ($p<0.1$, Table 1). IEM-1913 in a dose of 0.03 mg/kg decreased the incidence of pentylenetetrazole-induced tonic-clonic seizures by 4 times ($p<0.05$, Table 1). IEM-1913 in a dose of 0.1 mg/kg exhibited maximum anticonvulsant activity and decreased the incidence of tonic-clonic seizures by 8 times ($p<0.01$, Table 1). Hence, IEM-1913 was 2-fold more potent than selective NMDA receptor antagonists memantine and arcaine in decreasing the incidence of pentylenetetrazole-induced tonic-clonic seizures. As differentiated from memantine and arcaine, IEM-1913 in doses of 0.03-0.1 mg/kg

TABLE 1. Effect of Drugs on the Incidence of Pentylenetetrazole-Induced Clonic and Tonic-Clonic Seizures in Rats

Drug	Dose, mg/kg	Incidence of pentylenetetrazole-induced seizures, %	
		clonic	tonic-clonic
Distilled water (control)		100	80
Arcaine	1	95	70*
	5	85*	20+
Memantine	5	90	60*
	15	75*	20+
IEM-1913	0.01	65°	45°
	0.03	25+	20+
	0.1	20+	10x

Note. *$p<0.2$, +$p<0.05$, °$p<0.1$, and x$p<0.01$ compared to the control.

TABLE 2. Drug Safety (TD_{50}, ED_{50}, and Therapeutic Index)

Drug	TD_{50}, mg/kg	ED_{50}, mg/kg	Therapeutic index, TD_{50}/ED_{50}
Arcaine	25	2.5	10
Memantine	35	10	3.5
IEM-1913	30	0.015	2000

reduced the clonic component of pentylenetetrazole-induced seizures ($p<0.05$, Table 1).

Memantine and arcaine had low therapeutic index (TD_{50}/ED_{50}). Ataxia in 50% rats was observed after treatment with study drugs in doses (TD_{50}) that exceeded the effective anticonvulsant dose (prevention of tonic-clonic seizures in 50% rats, ED_{50}) only by 3.5-10 times (Table 2). The therapeutic index of IEM-1913 is 200-600 times higher than that of memantine and arcaine. Ataxia in 50% rats was observed after treatment with IEM-1913 in a dose (TD_{50}) that exceeded the effective anticonvulsant dose (ED_{50}) by 2000 times (Table 2).

As distinct from NMDA receptor antagonists, AMPA receptor antagonists completely prevent clonic and tonic-clonic seizures [6,8,15]. However, they have even lower therapeutic index. Prevention of pentylenetetrazole-induced seizures is observed only after administration of AMPA receptor antagonists in the maximum dose, which causes severe motor and behavioral disorders in rats [8,15]. Our study showed that mixed NMDA/AMPA receptor antagonist IEM-1913 not only prevents epileptogenesis of pentylenetetrazole-induced clonic and tonic-clonic seizures, but also has a higher therapeutic index than selective antagonists of NMDA and AMPA receptors.

Published data show that combined treatment with NMDA and AMPA receptor antagonists in low doses does not cause side effects and prevents kindled clonic and tonic-clonic seizures. They serve as a standard model for frontal temporal epilepsy in animals [7,9,11]. IEM-1913-like medicinal preparations with anti-NMDA/AMPA receptor activity hold much promise as a new generation of highly effective and low toxic anticonvulsant dugs for the therapy of generalized and local (clonic) seizures in patients with epilepsy.

REFERENCES

1. I. N. Abdurasulova, S. E. Serdyuk, and V. E. Gmiro, *Neiroimmunologiya*, No. 1, 4-11 (2007).
2. V. E. Gmiro and S. E. Serdyuk, *Eksper. Klin. Farmakol.*, **63**, No. 6, 3-8 (2000).
3. N. Ya. Lukomskaya, N. I. Rukoyatkina, L. V. Gorbunova, *et al.*, *Ros. Fiziol. Zh.*, **88**, No. 9, 1161-1171 (2002).
4. L. Bikjdaouene, G. Escames, E. Camacho, *et al.*, *J. Pineal Res.*, **36**, No. 4, 267-277 (2004).
5. Y. Feng, M. H. LeBlanc, and S. Regunathan, *Neurosci. Lett.*, **390**, No. 3, 129-133 (2005).
6. G. Ferreri, A. Chimirri, E. Russo, *et al.*, *Pharmacol. Biochem. Behav.*, **77**, No. 1, 85-94 (2004).
7. W. Loscher, *Prog. Neurobiol.*, **54**, No. 6, 721-741 (1998).
8. W. Loscher and D. Honack, *Br. J. Pharmacol.*, **113**, No. 4, 1349-1357 (1994).
9. W. Loscher and D. Honack, *Eur. J. Pharmacol.*, **259**, No. 2, R3-R5 (1994).
10. W. Loscher and B. Nolting, *Epilepsy Res.*, **9**, No. 1, 1-10 (1991).
11. W. Loscher, C. Rundfeldt, and D. Honack, *Eur. J. Neurosci.*, **5**, No. 11, 1545-1550 (1993).
12. K. Makarska-Bialek, R. M. Kaminski, and S. J. Czuczwar, *Pharmacol. Rep.*, **57**, No. 4, 458-466 (2005).
13. C. G. Parsons, G. Quack, I. Bresink, *et al.*, *Neuropharmacology*, **34**, No. 10, 1239-1258 (1995).
14. L. Velisek, S. Veresova, H. Pobisova, and P. Mares, *Psychopharmacology (Berl.)*, **104**, No. 4, 510-514 (1991).
15. H. Yamashita, K. Ohno, Y. Amada, *et al.*, *J. Pharmacol. Exp. Ther.*, **308**, No. 1, 127-133 (2004).