DOI 10.1007/s11055-015-0073-0
Neuroscience and Behavioral Physiology, Vol. 45, No. 3, March, 2015

Combined Blockade of AMPA and NMDA Receptors Produces Maximal Suppression of the Development of Corasol Kindling in Rats

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Translated from Rossiiskii Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 99, No. 5, pp. 612–618, May, 2013. Original article submitted December 27, 2012. Revised version received March 5, 2013.

Chronic enteral administration of the standard antiepileptic drug sodium valproate at a dose of 200 mg/kg suppressed the development of generalized clonic-tonic corasol-kindled convulsions in 100% of rats but prevented kindled clonic convulsions in only 57%. This dose of sodium valproate decreased the mean severity of corasol-kindled convulsions by a factor of 1.7. Chronic enteral administration of IEM-2121, which induces combined blockade of AMPA and NMDA glutamate receptors, and IEM-1676, which blocks AMPA and NMDA receptors, as well as n-cholinoreceptors, given at doses of 10 and 20 mg/kg, respectively, had greater anticonvulsive activity than sodium valproate, as they decreased the mean severity of corasol-kindled convulsions by factors of 2.4–2.7 as compared with controls and prevented kindled clonic convulsions in 87% of rats. Combined blockade of AMPA and NMDA receptors and, possibly, n-cholinoreceptors, produced the greatest suppression of epileptogenesis in relation to both clonic and clonic-tonic corasol-kindled convulsions.

Keywords: IEM-1676, IEM-2121, valproate, corasol, convulsions, kindling.

Corasol kindling is a widely used chronic preclinical model of temporal lobe epilepsy in rats, which is used for investigating substances for epileptogenesis and the generation of convulsions during the process of kindling [13, 14, 17]. Increased levels of endogenous glutamate and increased expression of NMDA and AMPA receptors in the hippocampus and cortex have been shown to play an important role in the development of corasol kindling [5–7].

Sodium valproate is a standard antiepileptic substance, chronic prophylactic administration of which in rats and mice produces maximal suppression of the development of generalized clonic-tonic corasol-kindled convulsions but prevents local clonic kindled convulsions in only some animals [8, 15].

The antiepileptic action of valproate has been shown to be based on activation of inhibitory GABAergic mechanisms due to increases in GABA synthesis, inhibition of GABA transaminase, and release of GABA from terminals, though valproate did not significantly reduce the toxic actions of glutamate on NMDA and AMPA receptors in kindling [12, 18].

At the same time, combined blockade of NMDA and AMPA receptors is known to produce complete suppression of the toxic action of glutamate and effectively not only blocks clonic-tonic, but also significantly decreases clonic kindled convulsions in a model of kindling induced by electrical stimulation of the amygdala in rats [9, 11].

We have synthesized the bis-cationic compounds IEM-2121 and IEM-1676, the therapeutic targets of which are glutamate NMDA- and AMPA-type ion channels. The IC_{50} values (the concentration at which the substance blocks 50% of open channels) for IEM-2121 were 3.4 μM for NMDA and 0.46 μM for AMPA receptors. IC_{50} values for IEM-1676 were 270 μM for NMDA and 8.8 μM for AMPA receptors [3]. Compound IEM-1676 is also a blocker of n-cholinoreceptors in ganglionic neurons. IEM-1676 has been found to have 1.5 times greater cholinolytic activity than the

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standard ganglion blocker hexonium in experiments on the superior cervical sympathetic ganglion in the cat and to have 60 times greater activity in the parasympathetic ganglion of the guinea pig [1].

It might be expected that IEM-2121, which evokes combined blockade of AMPA and NMDA receptors, and IEM-1676, which evokes combined blockade of AMPA and NMDA receptors and n-cholinoreceptors, would, on chronic administration, produce maximal suppression not only of tonic-clonic, but also clonic corasol-kindled convulsions.

The aim of the present work was to compare the efficacy of the anticonvulsant actions of the standard antiepileptic sodium valproate and agents IEM-2121 and IEM-1676 given by chronic enteral administration in rats with corasol kindling.

Methods

Experiments were performed on male Wistar rats weighing 180–200 g. On experimental day 1, rats were tested for sensitivity to single i.p. doses of corasol at the minimal effective dose of 60 mg/kg. Further studies were performed on rats which displayed clonic-tonic convulsions (severity 2–4 points) during the 30 min after administration of the test dose of corasol.

Corasol kindling was developed in the selected rats using i.p. corasol at the subconvulsive dose of 40 mg every other day from day 3 to day 21 of the experiment. Each dose of corasol was followed by observation for 30 min and recording of behavior. Convulsive manifestations were assessed on the Racine scale: 0 corresponded to the absence of a response, 1 to facial automatism and twitching of the ears and whiskers, 2 to convulsive waves propagating along the axis of the trunk, 3 to myoclonic convulsions with standing, 4 to clonic convulsions with loss of posture, 5 to repeated powerful clonic-tonic convulsions, and 6 to tonic convulsions leading to death [17]. Stage 1–3 convulsions were local clonic convulsions and stage 4–5 were generalized clonic-tonic seizures.

The effects of substances on epileptogenesis of kindled convulsions and the development of complete kindling were studied using a prophylactic scheme in which substances were given chronically before each corasol injection at the subconvulsive dose from day 3 to day 21 of the experiment [13, 17].

Animals received daily enteral IEM-2121 at doses of 0.3, 1.0, 3.0, and 10 mg/kg, IEM-1676 at doses of 1.0, 3.0, 10.0, and 20 mg/kg, as well as the standard antiepileptic sodium valproate at doses of 50, 100, and 200 mg/kg using a rigid metal intragastric tube, in 1 ml, 45 min before administration of corasol, from day 3 to day 21 of the experiment. Animals of the control group received intragastric doses of 1 ml of distilled water 45 min before administration of corasol from day 3 to day 21 of the experiment.

Throughout the experiment, mean convulsion severity was assessed in points for each dose of study substance in groups of 7–8 rats, along with the numbers and proportions of completely kindled rats and the numbers and proportions of rats without kindling (convulsion severity less than 2 points).

The anticonvulsant activity of test compounds and sodium valproate was assessed on kindling day 21, i.e., after completion of substance administration using the prophylactic scheme, in terms of decreases in the mean severity of kindled convulsions from the level seen in controls and in terms of decreases in the numbers of completely kindled rats and increases in the numbers of rats without kindling as proportions of control values.

Test compounds IEM-2121 (1-amino-6-(3,5-dimethyl-1-adamantylamino)-hexane dihydrochloride) and IEM-1676 (1-trimethylammonio-5-(1-adamantylammonio-pentane dibromide) were synthesized at the Institute of Experimental Medicine, Russian Academy of Medical Sciences, and standard reference agent sodium valproate was obtained from Sigma. Experimental data from the corasol kindling model were analyzed statistically using Fisher's test.

Results

In these experiments, repeated administration (7–11 injections) i.p. corasol at the subconvulsive dose of 40 mg/kg to control rats (daily intragastric distilled water 45 min before corasol) induced kindled convulsions of severity 3–5 points by experimental day 21 in all eight rats, while complete kindling (repeated generalized clonic-tonic convulsions of severity 4–5 points) occurred in six of the eight rats (75% of rats with complete kindling). The mean severity of kindled convulsions in control rats was 3.8 ± 0.9 points (see Table 1).

Chronic prophylactic enteral administration of the standard antiepileptic substance sodium valproate at a dose of 50 mg/kg produced virtually no decrease in the number of rats with complete kindling or in the mean severity of kindling seizures, as compared with controls (see Table 1). At the high doses of 100 and 200 mg/kg, chronic enteral sodium valproate decreased the mean severity of kindling convulsions by factors of 1.6–1.7, and reduced the numbers of completely kindled rats by 86 and 100%, respectively, ($p < 0.01$, see Table 1) as compared with controls. At these doses, sodium valproate significantly decreased the numbers of rats not showing kindling, by 43 and 57% compared with controls ($p < 0.05$, see Table 1).

Chronic enteral administration of compound IEM-2121 at the low dose of 0.3 mg/kg produced only a trend to reduction in the number of completely kindled rats and had essentially no effect on the mean severity of kindled convulsions compared with controls (see Table 1). IEM-2121 at a dose of 1 mg/kg significantly ($p < 0.05$) reduced the number of completely kindled rats by 32% from control, though the reduction in the number of rats not displaying kindling was by only 14% (see Table 1).

IEM-2121 at doses of 3 and 10 mg/kg decreased the mean severity of kindled seizures from the control level by factors of 1.8 and 2.4, respectively, and produced 86 and 100% reductions in the numbers of rats with complete kin-

TABLE 1. Effects of Study Agents on the Development of Corasol Kindling and the Severity of Kindled Seizures in Rats

Substance	Dose (intragastric), mg/kg	Total number of rats in group, *n*	Number of rats with complete kindling, % of total number of rats in group*	Number of rats without kindling, % of total number of rats in group**	Mean severity of kindled seizures, points***
Control (distilled water)		8	75	0	3.8 ± 0.9
Sodium valproate	50	7	71	0	3.7 ± 0.7
	100	7	14[1]	43[2]	2.3 ± 0.61[2]
	200	8	0[1]	57[2]	2.2 ± 0.51[2]
IEM-2121	0.3	7	57	0	3.5 ± 1.1
	1.0	7	43[2]	14	3.1 ± 0.9
	3.0	8	14[2]	57[2]	2.1 ± 0.6[2]
	10	8	0[1]	87[1]	1.6 ± 0.4[1]
IEM-1676	1	7	43[2]	14	2.9 ± 0.7
	3	8	14[1]	43[2]	2.1 ± 0.61[2]
	10	8	0[1]	71[1]	1.6 ± 0.52[1]
	20	8	0[1]	87[1]	1.4 ± 0.6[1]

Note. *Generalized clonic-tonic seizures, 4–5 points, three sequential doses of corasol at the subconvulsive dose of 40 mg/kg (i.p.); **clonic seizures, <2 points, kindling day 21; ***kindling day 21; [1]$p < 0.01$ compared with controls; [2]$p < 0.05$ compared with controls.

dling ($p < 0.01$, see Table 1). At these doses, IEM-2121 decreased the numbers of rats without kindling by 57 and 87% of control, respectively, ($p < 0.05$, $p < 0.01$, see Table 1).

Thus, IEM-2121 at a dose of 10 mg/kg produced the maximum possible anticonvulsant effect, as it suppressed the development of generalized clonic-tonic kindled convulsions in 100% of rats and prevented the development of kindled local clonic seizures of severity 2–3 points in 87%.

Chronic prophylactic enteral administration of compound IEM-1676 at the low dose of 1.0 mg/kg significantly ($p < 0.05$) decreased the number of rats with complete kindling by 32% from the control level, but decreased the number of rats without kindling by only 14% (see Table 1). IEM-1676 at doses of 3, 10, and 20 mg/kg significantly (by factors of 1.8, 2.4, and 2.7) decreased the mean severity of kindled seizures compared with controls (see Table 1). At these doses, IEM-1676 significantly decreased the number of completely kindled rats by 61–100% compared with controls ($p < 0.01$), with 43, 71, and 87% decreases, respectively, in the numbers of rats without kindling ($p < 0.05$, $p < 0.01$, see Table 1).

Thus, IEM-1676 at a dose of 20 mg/kg induced the maximum possible anticonvulsant effect, as it suppressed the development of generalized clonic-tonic kindled seizures in 100% of rats and prevented the development of clonic kindled seizures in 87%.

Chronic prophylactic administration of sodium valproate in rats and mice produced the maximum suppression of the development of generalized clonic-tonic corasol-kindled seizures, though it prevented local clonic kindled seizures in only a proportion of the animals [8, 15]. It can be suggested that sodium valproate suppresses epileptogenesis of generalized kindled convulsions due to increases in GABAergic inhibition of pyramidal and cortical neurons, though valproate has no significant influence on the epileptogenesis of kindled clonic convulsions, as it does not weaken the toxic actions of glutamate on NMDA and AMPA receptors [12, 18].

The present results support the view that prophylactic enteral sodium valproate at doses of 100–200 mg/kg produces the maximum suppression of the development of kindled generalized clonic-tonic kind of seizures (in 86–100% of rats), but prevents the development of clonic kindled seizures in only 43–57%.

Chronic administration of NMDA receptor blockers (MK-801 and memantine), as well as the AMPA blocker CFM-2, before each dose of corasol at the subconvulsive dose is known to produce significant slowing of the development of kindling, to decrease the severity of kindled seizures, and to prevent the development of repeated generalized clonic-tonic seizures in completely kindled rats, though selective blockade of NMDA or AMPA receptors is insufficient to prevent kindled local clonic seizures [2, 4, 9]. At the same time, combined blockade of NMDA and AMPA receptors completely suppresses the toxic actions of glutamate and effectively not only eliminates clonic-tonic seizures, but also significantly weakens kindled clonic seizures in another model of kindling, induced by electrical stimulation of the amygdala in rats [9, 11].

IEM-2121 has been shown in experiments on brain slices to have combined NMDA- and AMPA-blocking activity, while IEM-1676 blocks AMPA and NMDA receptors, as well as n-cholinoreceptors [1, 3]. Comparative analysis of the chronic enteral anticonvulsive activities of these com-

pounds and sodium valproate demonstrated that IEM-2121 (10 mg/kg) and IEM-1676 (20 mg/kg) were significantly more active than sodium valproate in a corasol kindling model in rats, as they decreased the mean severity of kindled seizures 1.5–1.6 times as effectively as sodium valproate and prevented kindled clonic seizures in 87% of rats, as compared with 57% after use of sodium valproate.

The epileptogenesis of kindled convulsions is known to be based on combined stimulation of NMDA and AMPA receptors by endogenous glutamate [2, 4, 6, 7, 9, 16]. The results of these experiments lead to the conclusion that combined blockade of AMPA and NMDA receptors induced by IEM-2121 produces maximal suppression of the epileptogenesis of not only generalized clonic-tonic seizures, but also of kindled local clonic seizures.

IEM-1676, like IEM-2121, is a powerful blocker of AMPA receptors, though unlike IEM-2121, it also has weak NMDA-blocking activity [3]. At the same time, IEM-1676 had high n-cholinolytic activity [1].

Loscher et al. [10] have demonstrated that substances producing combined blockade of NMDA receptors and n-cholinoreceptors have stronger anticonvulsive effects in an electroshock model in mice than substances selectively blocking only one of these receptors. It can therefore be suggested that IEM-1676 should yield maximal suppression of both clonic-tonic and clonic seizures kindled by corasol as a result of the combined blockade of not only AMPA and NMDA receptors, but also n-cholinoreceptors.

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