Effect of Modulators of the Polyamine Site on the Development of Seizures Induced by Systemic and Intracerebral Administration of N-Methyl-D-Aspartate in Albino Mice

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Systemic intraperitoneal administration of polyamine agonist IEM-1460 containing the Me_3N^+ group with a stable positive charge preventing permeation of this substance through the blood-brain barrier and polyamine antagonist arcaine had no effect on the development of seizures caused by intracerebral injection of N-methyl-D-aspartate in mice. Intraperitoneal injection of IEM-40 potentiated, while arcaine decreased the severity of seizures induced by intraperitoneal treatment with N-methyl-D-aspartate. This effect was related to modulation of the permeability of the blood-brain barrier for N-methyl-D-aspartate probably due to modulating effects of IEM-40 and arcaine on the polyamine site of N-methyl-D-aspartate receptors in the blood-brain barrier.

Key Words: *1-trimethylammonio-5-(1-adamantanemethylammonio)-pentane dibromide; spermine; arcaine; N-methyl-D-aspartate; seizures*

Antagonists of the polyamine site of N-methyl-D-aspartate (NMDA) receptors arcaine and ifenprodil in systemic administration are not inferior to NMDA receptor channel blockers memantine and MK-801 by their anticonvulsant activity in seizures modeled by systemic administration of NMDA and pentylenetetrazole and depending on permeability of the blood-brain barrier (BBB) [1,2,7,12].

However, systemic administration of polyamine antagonists, in contrast to MK-801 and memantine, is low effective during electric shock-induced or audiogenic seizures, which do not depend on BBB permeability [5,6]. Since polyamine antagonists after peripheral administration decrease BBB permeability [4,9], it can be expected that intraperitoneal injection of arcaine would reduce the severity of seizures induced by intraperitoneal injection of NMDA due to a decrease in BBB permeability for NMDA, but will not modulate the severity of seizures induced by intracerebral administration of NMDA not depending on BBB permeability.

Polyamine agonist spermine after intracerebral administration increases the severity of seizures induced by systemic treatment with NMDA and potentiates the neurotoxic effect of NMDA and glutamate in brain slices due to activation of the polyamine site of NMDA receptors in the brain [3,8]. Previous studies showed that systemic administration of spermine considerably aggravates seizures caused by peripheral and intracerebral administration of NMDA and increases animal mortality rate [1,12]. Since systemic administration of spermine increases BBB permeability for polar compounds [10,11], potentiation of seizures induced by peripheral administration of NMDA is associated with an increase in BBB permeability and direct activation of the NMDA receptor polyamine site in the brain due to high permeability of BBB for spermine.

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IEM-1460, a selective antagonist of glutamate Ca^{2+}-permeable AMPA receptors, was synthesized in the Institute of Experimental Medicine. EM-1460 exhibits properties of a polyamine agonist under *in vivo* conditions and in hippocampal slices [1]. We hypothesized that intraperitoneal injection of polyamine agonist IEM-1460 will potentiate seizures induced by intraperitoneal treatment with NMDA due the increase in BBB permeability for NMDA, but, in contrast to spermine, will not modulate the severity of seizures induced by intracerebral administration of NMDA, because the positive charge of the Me_3N^+ group prevents permeation of IEM-1460 through BBB into the brain.

Here we studied the effectiveness of a new approach for modulation seizures induced by systemic administration of NMDA in a toxic dose via modulation of BBB permeability for polar compounds after peripheral administration of agonists and antagonists of the NMDA receptor polyamine site. To this end we compared the effects of intraperitoneal treatment with polyamine antagonist arcaine and IEM-1460 on the incidence of seizures induced by intraperitoneal and intracerebral administration of NMDA. Moreover, we compared the mortality rates under these conditions.

MATERIALS AND METHODS

Experiments were performed on 136 male mice weighing 18-20 g. NMDA (Sigma) was injected intraperitoneally and centrally (into the lateral ventricle) in convulsant doses of 200 mg/kg and 0.5 μg/5 μl, respectively. NMDA was administered intracerebroventricularly via a stereotaxic injector [14]. Polyamine site agonist 1-trimethylammonio-5-(1-adamantanemethylammonio)-pentane dibromide (IEM-1460) was injected intraperitoneally in a dose of 1 mg/kg [1]. Polyamine antagonist arcaine (Sigma) was injected intraperitoneally in doses of 5 and 10 mg/kg. The test compounds were administered 20 min before NMDA treatment.

The incidence of tonic-clonic seizures and mortality rate in each group (percent of the total number of animals) were recorded over 30 min after NMDA administration. In experiments with central administration of NMDA, permeation of the drug into the lateral cerebral ventricle was verified. The results were analyzed by Fischer exact test.

RESULTS

Systemic intraperitoneal administration of NMDA in a dose of 200 mg/kg induced tonic-clonic seizures in 70% mice. The mortality rate reached 40% (Table 1). Intracerebroventricular administration of NMDA in a dose of 0.5 μg induced seizures in 62% mice. Under these conditions, the mortality rate was 14% (Table 2).

Systemic intraperitoneal administration of arcaine in a dose of 5 mg/kg significantly decreased the mortality rate and incidence of seizures induced by intraperitoneal injection of NMDA (by 4 and 7 times, respectively; Table 1). However, intraperi-

TABLE 1. Effect of Polyamine Modulators on Convulsant Activity and Mortality Rate of Mice after Intraperitoneal Injection of 200 mg/kg NMDA

Substance	Seizures, %	Mortality rate, %
NMDA (control)	70	40
IEM-1460 (1 mg/kg)+NMDA	70	70
Arcaine (5 mg/kg)+NMDA	10*	10
Arcaine (10 mg/kg)+NMDA	30	30
Arcaine (5 mg/kg)+IEM-1460 (1 mg/kg)+NMDA	60+	60+
Arcaine (10 mg/kg)+IEM-1460 (1 mg/kg)+NMDA	30	30

Note. *$p<0.01$ compare to the control; +$p<0.05$ compared to arcaine (5 mg/kg)+NMDA.

TABLE 2. Effect of Polyamine Modulators on Convulsant Activity and Mortality Rate of Mice after Central Administration of NMDA (0.5 μg, intracerebroventricularly)

Substance	Seizures, %	Mortality rate, %
NMDA (control)	62	14
IEM-1460 (1 mg/kg)+NMDA	50	20
Arcaine (5 mg/kg)+NMDA	62.5	12.5
Arcaine (5 mg/kg)+IEM-1460 (1 mg/kg)+NMDA	75	12.5

toneal injection of arcaine did not decrease the severity of seizures induced by intracerebral administration of NMDA (Table 2). Published data show that systemic and intracerebral administration of a polyamine antagonist ifenprodil is low effective during electric shock-induced or audiogenic seizures not depending on BBB permeability [5,6], whereas in NMDA-induced seizures, systemic administration of ifenprodil, similarly to arcaine, prevents the development of seizures [7,12]. Hence, despite high permeability of BBB for arcaine and ifenprodil, systemic treatment with these compounds has only a peripheral anticonvulsant effect and is not related to blockade of the NMDA receptor polyamine site in mouse brain during seizures induced by intraperitoneal injection of NMDA. Since polyamine antagonists after peripheral administration decrease BBB permeability for polar compounds [4,10], it can be hypothesized that arcaine prevents the development of seizures induced by intraperitoneal injection of NMDA due to a decrease in BBB permeability for NMDA, which is associated with the blockade of the NMDA receptor polyamine site in BBB.

Systemic administration of NMDA receptor polyamine site agonist spermine increases the mortality rate from seizures induced by peripheral and intracerebral treatment with NMDA [1,12]. Since systemic administration of spermine increases BBB permeability for polar compounds [10,11], potentiation of seizures induced by peripheral administration of NMDA can be determined by both increased BBB permeability for NMDA and direct activation of the NMDA receptor polyamine site in the brain due to high permeability of BBB for spermine.

IEM-1460 exhibits properties of a polyamine agonist under *in vivo* conditions and in hippocampal slices [1]. Intraperitoneal injection of spermine in a dose of 1 mg/kg 2-fold increased animal mortality rate during seizures induced by intraperitoneal treatment with NMDA (Table 1), but had no effect on the severity of seizures induced by intracerebral administration of NMDA (Table 2). Since IEM-1460 little crosses BBB, it can be hypothesized that systemic administration of this compound potentiates seizures induced by systemic injection of NMDA only by increasing BBB permeability for NMDA (in contrast to spermine).

IEM-1460 (1 mg/kg) abolished the inhibitory effect of arcaine (5 mg/kg) on seizures induced by peripheral administration of NMDA (Table 1). Increasing the dose of arcaine to 10 mg/kg not only abolished the anticonvulsant effect of IEM-1460, but also decreased the incidence and mortality rate from seizures induced by peripheral administration of NMDA (Table 1). Combined intraperitoneal administration of IEM-1460 and arcaine had little effect on the incidence and mortality rate from seizures induced by central treatment with NMDA (Table 2). Hence, arcaine and IEM-1460 are characterized by only peripheral competitive antagonism during seizures induced by systemic administration of NMDA. These specific features are probably related to the opposite modulatory effect of the test compounds on the NMDA receptor polyamine site in BBB.

Our results indicate that systemic administration of peripheral polyamine agonist IEM-1460 potentiates seizures induced by systemic treatment with NMDA by increasing BBB permeability for NMDA determined by the activating effect of IEM-1460 on the NMDA receptor polyamine site in BBB.

These findings demonstrate high effectiveness of a new approach to modulate the severity of seizures induced by systemic administration of a convulsant agent. Variations in BBB permeability for polar compounds are associated with the modulatory effect of arcaine and IEM-1460 on the NMDA receptor polyamine site in BBB.

The proposed method for peripheral regulation of convulsant activity in the brain is more safe that standard methods for central regulation, because IEM-1460 and arcaine has no toxic effect on the central nervous system.

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