

Metabotropic glutamate receptor 5 antagonist, MPEP, lacks anticonvulsant activity in acute models of epilepsy

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Abstract. Evaluation of the effects of different doses of 2-methyl-6-phenylethynyl-pyridine (MPEP), a selective antagonist of mGluR5, in acute models of epilepsy [pentylentetrazole (PTZ) and maximal electroconvulsive shock (MES)], dosage relations and also whether if this effect is enhanced by conventional antiepileptic drugs. The anticonvulsant properties of MPEP were examined in mice who received MPEP by intracerebroventricular infusion prior to a subcutaneous injection of pentylentetrazole and maximal electroshock. Evaluations of convulsive seizures caused by PTZ were performed by the loss of righting reflex while convulsive seizures caused by MES were performed by the occurrence of tonic hind limb extension in adult mice. There was no significant difference in both groups (PTZ, MES) when compared with the control group. The present results do not support a significant anticonvulsant potential of MPEP on adult mice as a selective antagonist of mGluR5 in convulsive seizures.

Key words: metabotropic glutamate receptor 5, 2-methyl-6-phenylethynyl-pyridine, convulsive seizures

1. Introduction

One of the mechanisms of the antiepileptic drugs is receptor antagonism or prevention of glutamate release. The neurotoxic side effects of ionotropic glutamate receptor antagonists have addressed the studies to metabotropic glutamate receptors (mGluRs) that have fewer side effects (1).

The identification of subtype-selective agonists and antagonists has directed the investigations to mGluRs as potential targets in the treatment of epilepsy. Metabotropic glutamate receptors consist of eight subtypes that were divided into three subgroups on the basis of their amino acid sequence identities, pharmacological profiles and signal transduction pathways. Group I mGluRs (i.e. mGluR1 and mGluR5) are coupled to the phosphoinositide-calcium cascade, while group II mGluRs (mGluR2 and mGluR3) and group III

mGluRs (mGluR4, mGluR6, mGluR7 and mGluR8) are linked to inhibition of adenylate cyclase via pertussis toxin sensitive G-proteins (2,3). Activation of group II or group III mGluRs has been established to be neuroprotective in vitro and in vivo. In contrast, group I mGluRs need to be antagonized in order to evoke protection (4).

The roles of mGluR antagonists were examined in modulation of seizures activity in vitro studies. In vivo data from animal models of epilepsy have shown modulator roles of mGluRs on ictal activity during seizures (5,6). The identification of 2-methyl-6-phenylethynyl-pyridine (MPEP), a highly selective and brain-penetrant mGluR5 antagonist, allowed the investigations of the therapeutic potential of this class of Ligand (7).

Both subtypes of group I mGluRs contribute to produce prolonged discharges and could be associated with additive effects of mGluR1 and of mGluR5 antagonists (8). mGluR1 and mGlu5, are concentrated in hippocampus, and two subtypes have different intracellular calcium responses (9). Both receptor subtypes participate in the induction and maintenance of mGluR-mediated burst prolongation, mGluR1 activation plays a greater role in sustaining the expression of prolonged bursts, whereas mGluR5 activation may be a more critical contributor to the

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Table 1. PTZ-induced convulsive models

Treatment	Mice (n)	Duration of loss of righting reflex (min)	Mice having no seizure activity (n)	Death (n)	p*
Saline	8	9.6 ± 1	0	5	
MPEP 1 µg	8	8.4 ± 2.1	0	3	0.886
MPEP 10 µg	20	11.1 ± 2.7	0	10	0.685
MPEP 50 µg	17	9.7 ± 1.3	0	5	0.340
Ethosuximide 150 mg/kg	8	60	8	0	
Ethosuximide 50 mg/kg	8	15.3 ± 4.4	0	0	0.05
Ethosuximide 50 mg/kg + MPEP 10 µg	8	16.3 ± 3.8	0	0	0.01

*Statistical significance in comparison to control group

induction process underlying the epileptogenesis (10).

In this study, we aimed to evaluate the effects of different doses of MPEP, a selective antagonist of mGluR5, in acute epilepsy models (PTZ and MES), dosage relations and also whether this effect is enhanced by conventional antiepileptic drugs.

2. Materials and methods

2.1. Animals and materials

Male, adult Balb/C mice weighing 25-35 g were obtained from Gulhane Military Medical Academy Research and Development Laboratory. The animals were housed in a temperature-controlled room (20-22°C) with a 12-h light/dark cycle and provided with food and water ad libitum. All experiments have been done at daylight period and at the same time period. Procedures described above were approved by Gulhane Military Medical Academy Animal Care and Use Committee in accordance with The Guide for the Care and Use of Laboratory Animals.

MPEP and ethosuccimide were purchased from Sigma Chemical Lab., USA and PTZ was supplied from Ibrahim Ethem Lab., Turkey. Diethyl ether was obtained from Gulhane Military Medical Academy Medical Pharmacology Department.

2.2. Intracerebroventricular Application of MPEP

Male, adult Balb/C mice were anesthetized with diethyl ether and scalps had been incised 8 mm starting from 2 mm behind the midline of intersection point of eyes posterior region. After resection of soft tissues, “bregma” point was figured out. At the 2 mm lateral of bregma, the drugs were infused intracerebroventricular (i.c.v.) by 50 mm Hamilton injector (11).

2.3. Determination of PTZ-induced and MES-induced seizures

Recovery latency for righting reflex and death ratio were investigated in mice stimulated with PTZ (85 mg/kg) and MES (50 mA and 0.4 s) groups. The study groups were designed as 8 groups; subjects were divided into seven groups according to MPEP dosage and add-on therapy in PTZ-induced convulsive model (Table 1). The first group was control group who received 10 µL saline. 1, 10, 50 µgr MPEP were applied by i.c.v. infusion to the second, third and fourth groups in PTZ-induced convulsive model respectively. Ethosuccimide (150 mg/kg) was applied intraperitoneally to the fifth group to prevent rats from myoclonic seizures. Ethosuccimide (50 mg/kg) was applied in the sixth group and ethosuccimide (50 mg/kg) + 10 µgr MPEP groups were formed in the seventh group to determine

Table 2. MES-induced convulsive models

Treatment	Mice (n)	Mice having seizure activity (n)	Mice having no seizure activity (n)	Death (n)
Saline	8	8	0	0
MPEP 50 μ g	8	8	0	0

the potentiation effect of MPEP. The eighth group was the only MES-induced convulsive model; 50 μ g MPEP were injected and applied 50 mA shocks during 0.5 seconds via the ear-clip electrodes after 15 minutes (table 2). Mice were observed for the occurrence of tonic hind limb extension taken as the endpoint. All test compounds were solubilized in saline and the pH was adjusted to 6-7.

Following PTZ injection, mice were observed for the acute behavioral signs of spontaneous convulsive activity, during 15 minutes. At 15 min following i.c.v. infusion of test compound, a subcutaneous bolus of PTZ (85 mg/kg) was administered. Convulsive activities in mice were evaluated using two parameters.

Righting reflex (Falling of the rat at right or left by losing the muscle tone of 4 extremities).

Death rate was evaluated.

2.4. Statistical analysis

SPSS[®] for Windows[®] (SPSS Inc., Chicago, IL, USA) was used to analyse the results. Kruskal-Wallis analysis of variance and the Mann-Whitney U test were used to compare the data between groups. The survival probabilities of the groups were calculated by Kaplan-Meier test. $p < 0.05$ was considered as statistically significant.

3. Results

Concerning the recovery latency for righting reflex, no marked statistical differences were observed at doses of 1, 10, 50 μ g MPEP ($p=0.935$). While the shortest mean time was found in 1 μ g MPEP group (8.36 ± 2.13 min), the 50 mg/kg ethosuccimide + 10 μ g MPEP group exhibited the longest duration of loss of righting reflex (16.2 ± 3.8 min). In 150 mg/kg ethosuccimide group, there was no convulsion or death. In 50 mg/kg ethosuccimide group, the time for loss of righting reflex was 15.8 ± 4.4 min. In 50 mg/kg ethosuccimide + 10 μ g MPEP group, there was no death and duration of loss of righting reflex was 16.3 ± 3.8 min. As shown in Table 2, MPEP did not affect PTZ-induced seizures latency with ethosuccimide to observe the potentiating; comparing 50 mg/kg

ethosuccimide group with 50 mg/kg ethosuccimide + 10 μ g MPEP group, there was no statistically difference between the duration of loss of righting reflex ($p=0.295$). While ethosuccimide was able to block the seizures with anticonvulsant dose, no effect was observed when co-infused with MPEP on seizures (table 1).

There were dead subjects in first 4 groups, there was no statistically meaningful difference between the first control group and different doses of MPEP groups according to the survival rates; between group1 and 2 [log rank=0.02 ($p=0.886$)], group1 and 3 [log rank = 0.16 ($p=0.685$)], group1 and 4 [log rank=0.91 ($p=0.340$)]. In ethosuccimide group, there was no death and the highest death rate was seen in saline group (62.5%).

There was no death in MES convulsive model, whereas all the subjects had seizures, suggesting that MPEP didn't have functional activity on MES induced-seizures (table 2).

4. Discussion

Physiological and pathophysiological processes of the central nervous system were rather limited, since the first generation of selective antagonists described for mGluRs. Phenyl glycine derivatives were widely examined in model systems of neurodegeneration and neuroprotection. The second generation of group I mGluRs antagonists show similar profile without any activity at mGluR5 and aminoindan-1,5 dicarboxylic acid, dose-dependently antagonized PTZ-induced seizures, is a mGluR1-selective antagonist with little activity at mGluR2, 4, and 5. (3,4,12,13). Two-methyl-6-phenylethynyl-pyridine is the first highly potent, noncompetitive and selective mGlu5R antagonist (7).

Metabotropic glutamate receptor modulators were proposed to play a role in epileptogenesis as an antiepileptic agent in a number of animal seizure models. We have analyzed the role of mGlu5R activity in convulsive seizures by using MPEP at different doses to find out clinical response and to observe the efficacy as an add-on therapy on both chemically and MES-induced epilepsy models. In the present study, we've demonstrated that i.c.v. infusion of MPEP up to a

dose of 50 µgr had no marked anticonvulsive effect on PTZ and MES convulsive models. Nagaraja RY et. al. (14). reported that MPEP could have efficacy against convulsive seizures induced by lower doses of PTZ (40 mg/kg), it was ineffective in counteracting seizures evoked by higher PTZ doses. When MPEP was used at different concentrations in acute seizures, we didn't find any mGlu5R response to PTZ induced seizures at 85 mg/kg in compatible with this study. Our negative results with MPEP may accomplish with the idea that blockage of mGluR5 alone is not adequate for anticonvulsive effect, but mGluR1 together with mGluR5 may play a greater role in epileptogenesis (9,15). One of the most common animal seizure models in developing antiepileptic drugs is the MES model especially specific for creating generalized tonic-clonic contractions and predictive value of determining clinically effective antiepileptic is high (16,17). Another popular acute contraction model is PTZ contraction test that was used in the development of drugs in nonconvulsive and myoclonic seizures (17-19). In order to clarify the contribution of the potency of mGluR5 in acute convulsions, we used both PTZ and MES convulsive tests to find out efficacy of MPEP in any type of acute seizure models. Suzuki et al. examined the roles of 3 types of mGluRs (I, II, III) and suggested that group 1 mGluR may play a considerable role in PTZ induced seizures on rats during diazepam withdrawal (20). Barton ME et. al. reported mGluR5 was effective in the MES model (16). In contrast to these studies, our work showed that MPEP didn't prevent the mice from neither PTZ model with myoclonic seizures nor MES model with generalized tonic contractions. Loscher W et. al. (21) illustrated ineffective anticonvulsant activity of group I mGluR antagonists in rodent models of difficult-to-treat partial epilepsy. Mares P et al (22). suggested that MPEP exhibits anticonvulsant action in immature rats but without a serious impairment of motor performance as we showed by evaluating righting reflex. Recent data point out the activation of mGluR5 may play a triggering role in the initiation of seizures. MPEP was moderately effective in suppressing maintenance of the potentiated bursts and markedly effective in preventing the induction of long-lasting prolonged burst in kindling models (9). Although acute convulsion tests have the advantage of testing many antiepileptic agents, evaluation of mGluR5 antagonists in kindling type of chronic epilepsy models may give brighter information.

Ethosuccimide is clinically used as a classic anticonvulsant drug. We observed the efficacy in PTZ models as well but MPEP in combination with ethosuccimide with reduced dose was not associated with anticonvulsant interaction or potentialization affect on anticonvulsant activity. This result also amplified the idea of antagonism of mGluR5 is not sufficient in acute seizures even co-administered with classic antiepileptics.

In summary, we examined MPEP at PTZ and MES models. The present results showed us that anticonvulsant potential of mGluR5 and add-on therapy lack in acute convulsive models by evaluating righting reflex and tonic hind limb extension. We thought that mGluR5 antagonists might represent an exciting new therapeutic agent for the treatment of psychiatric and neurological disorders, including anxiety and depression, addiction, Parkinson's disease, and inflammatory pain (23). Selective mGluR5 antagonists on kindling type of chronic epilepsy models may give encouraging information.

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