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Original Article

Amelioration of Pentylenetetrazole-Induced Seizures by Modulators of Sigma, N-Methyl-D-Aspartate, and Ryanodine Receptors in Mice

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Abstract

Background: Sigma receptors, N-methyl-D-aspartate (NMDA) antagonist, and modulators of intracellular calcium may be useful for seizure control. Therefore, we aimed to evaluate the antiepileptic effects of opipramol, a sigma receptor agonist, against pentylenetetrazole (PTZ)-induced seizures in mice and assess ketamine and caffeine interaction with the antiepileptic effects of opipramol.

Methods: PTZ (100 mg/kg) was used for the induction of seizure in 72 male albino Swiss strain of mice (n=8). Opipramole (10, 20, and 50 mg/kg), ketamine (50 mg/kg), caffeine (200 mg/kg), opipramole (20 mg/kg) plus ketamine (50 mg/kg), opipramole (20 mg/kg) plus caffeine (200 mg/kg), diazepam (5 mg/kg as a positive control), and the vehicle were administered interaperitoneally 30 minutes before the injection of PTZ. The latency was recorded for the clonic, tonic-clonic seizures, and death of animals after the injection of PTZ. Kruskal-Wallis test followed by Dunn's test was used for the analysis of data. Statistical analysis was performed with the SPSS software version 23.0 and $P<0.05$ was considered as the significant level.

Results: Opipramol (20 mg/kg) increased the latency for the PTZ-induced clonic (44%, $P=0.021$) and tonic-clonic (130.80%, $P=0.043$) seizures compared with the vehicle-treated group. Animals treated with opipramol (20 mg/kg) plus caffeine (200 mg/kg) had a significantly higher onset of PTZ-induced clonic and tonic-clonic seizures compared with the control ($P=0.046$ and <0.001, respectively). Ketamine combined with opipramol increased the onset of tonic-clonic seizure compared with the vehicle-treated groups ($P<0.001$).

Conclusion: Opipramol attenuated the seizures induced by the PTZ. Ketamine and caffeine had no effect on the anticonvulsant activity of opipramol.

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Keywords ● Opipramol ● Sigma ● Pentylenetetrazole ● Ketamine ● Caffeine

What's Known

- Sigma receptors may have roles in the seizure pathophysiology and sigma receptor modulators have produced anticonvulsant effects in the animal models of epileptic seizure.

What's New

- Opipramol, a sigma receptor agonist, exerted anticonvulsant effect in the pentylenetetrazole-induced seizures in mice. The interaction of opipramol with ketamine and caffeine had no effect on the anticonvulsant effects of this drug.

Introduction

Epilepsy is considered as the second most prevalent neurological disorder affecting more than 50 million people around the world.[1] Pharmacotherapy is the principal method for treating seizure disorders. Despite the successful treatment of patients with

currently available antiepileptic drugs, between 20-30% of the patients do not benefit from pharmacotherapy mainly because of resistance to the treatment or experiencing serious side effects after using antiepileptic drugs.[2,3] Therefore, there is a pressing need to find more efficacious or less toxic drugs to circumvent the limitations of currently available drugs.

Although the exact pathophysiology of epileptic seizure is not fully elucidated, it has been proposed that the imbalance between the excitatory and the inhibitory neurotransmission as well as deregulated intracellular calcium in the central nervous system (CNS) may be responsible for these disorders.[4,5] N-methyl-D-aspartate (NMDA) and ryanodine receptors are important cellular mechanisms that substantially contribute to the modulation of intracellular calcium. NMDA receptors are ionic channels highly permeable to calcium and increase intracellular calcium in neurons.[6] Moreover, ryanodine receptors are considered as caffeine-sensitive calcium stores that regulate calcium mobilization from intracellular pools.[7] Thus, the effects of NMDA or ryanodine receptors modulators like ketamine and caffeine on the intracellular calcium concentration can be considered as potential targets for the seizure control.

Intraperitoneal (IP) administration of pentylenetetrazole (PTZ) is one the most popular method for the screening and rational drug design of antiepileptic drugs.[8] The primary mechanism of PTZ is the antagonistic effects on the $GABA_A$ receptors. Moreover, the activation of NMDA receptors and increase in the intracellular calcium may contribute to the PTZ-induced seizures.[9,10] Therefore, agents that block NMDA receptors or modulate intracellular calcium may be effective against the PTZ-induced seizure and potentially valuable for the development of antiepileptic drugs.

Sigma receptors are chaperon proteins located on the sarcoplasmic reticulum,[11] which may have important roles in the modulation of intracellular calcium by interaction with NMDA glutamate receptors.[11,12] The exact roles of the sigma receptors in the CNS have not been fully elucidated yet. However, it has been shown that these receptors may be essential for the normal activity and survival of neurons in certain physiological and pathological conditions like epilepsy.[13,14] Some sigma-1 receptor modulators such as dextrorphan, carbetapentane, and pentazocine could attenuate seizures induced by the kainic acid or maximal electroshock in mice.[15-17] Moreover, Thurgur and Church[18] showed that at micromolar concentrations, a sigma-1 receptor agonist had antiepileptic activity in the rat hippocampal slices. With regard to the sigma receptors interaction with NMDA receptors and intracellular calcium, it is possible to assume that the modulators of NMDA and ryanodine receptors like ketamine and caffeine may affect antiepileptic effects of sigma receptor agonists.

Opipramol is a centrally acting drug approved for depression and anxiety treatment in some European countries.[19] Although opipramol belongs to the tricyclic antidepressants, it shows higher affinity for the sigma receptors, particularly the sigma-1 subtype[20] and minimal effects on the dopamine and phencyclidine receptors.[21,22] It has been revealed that opipramol exerts neuroprotective effects in an animal model of ischemia-induced neuronal loss.[22] However, there are very limited data in the literature about the potential roles of this agent in the treatment of seizure. Therefore, the aim of the present study was to evaluate the antiepileptic effects of opipramol, as a sigma receptor agonist, against PTZ-induced seizures in mice. Moreover, we aimed to show ketamine, an NMDA receptor antagonist, and caffeine, a modulator of intracellular calcium, interaction with the antiepileptic effects of opipramol in the PTZ-induced seizures.

Materials and Methods

Animals and Treatments

Male albino Swiss strain of mice weighing 20-40 g was provided by Razi Institute (Tehran, Iran) and housed on a regular dark/light cycles (12 h/12 h), controlled temperature (22±2°C), and free access to food and water in groups of 5 animals in plexiglass cages. A total of 72 mice wererandomly allocated to the 9 separate groups (n=8). We used opipramole (Sigma, USA) (10, 20, and 50 mg/kg), ketamine (Sigma, USA) (50 mg/kg), caffeine (Merck, USA) (200 mg/kg), opipramole (20 mg/kg) plus ketamine (50 mg/kg), opipramole (20 mg/kg) plus caffeine (200 mg/kg), diazepam (Daru Pakhsh, Iran) (5 mg/kg as a positive control) and the 30 minutes before the PTZ injection. All compounds administered 30 minutes before the injection of PTZ by the IP route. We prepared solutions on the basis of weight/volume and used 0.1 ml/10 g of the animal body weight. The experiment was approved by the Animal Ethics Committee of Bushehr University of Medical Sciences, which is in accordance with the European Communities Council to minimize the number and suffering of animals.

PTZ-Induced Seizure

We used PTZ (100 mg/kg) for the induction of clonic and tonic-clonic seizure in mice. After the injection of PTZ, animals moved into a separate cage and monitored for 30 minutes. The clonic seizure was defined as an over 3-second clonus of the animal body which is accompanied with the loss of righting reflex.[23] We recorded the latency for the clonic and generalized tonic-clonic seizures. Moreover, we recorded the latency of death of animals after injection of PTZ and the number of animals protected from PTZ-induced seizure and death. We measured seizure intensity according to the scale mainly depicted from Ali et al.[24] The scale was as follow: (0) without any body movement, (1) facial and body twitches, (2) nodding and body twitching, (3) clonus of the forelimb, (4) rearing, dropping on the floor, hindlimb clonus, and tonus of forelimb, (5) tonic-clonic seizure, status epilepticus, and/or death.

Data Analysis

Data were expressed as mean±standard error of mean (SEM) for the latency of clonic and generalized tonic-clonic seizures. We used the Kruskal-Wallis test followed by Dunn's test for the analysis of the onset of clonic, generalized tonic-clonic seizures, and the intensity of seizures. We used the Fisher's exact test to evaluate the number of animals protected from PTZ-induced death. The significant level was considered as $P<0.05$. Statistical analysis was performed by the SPSS software version 23.0.

Results

Effects of Different Treatments on the PTZ-Induced Clonic Seizure

The Kruskal-Wallis test showed that the onset of PTZ-induced clonic seizure was different between treatment groups ($X^2(8)=45.85$, $P<0.001$). Opipramol at a dose of 20 mg/kg increased the latency for the PTZ-induced clonic seizure compared with the vehicle-treated group (table 1). The latency for the clonic seizure in the animals treated with diazepam was significantly higher than the control groups (table 1). Furthermore, animals treated with opipramol (20 mg/kg) plus caffeine (200 mg/kg) or ketamine (50 mg/kg) had a significantly higher onset of PTZ-induced seizure compared with the vehicle-treated groups (table 1). However, opipramol at doses of 10 and 50 mg/kg and ketamine and caffeine alone had no effect on the onset of PTZ-induced clonic seizure in mice (table 1). Our study showed that the onset of clonic seizure in the opipramol combined with ketamine group was not significantly different from opipramol or ketamine treated groups (table 1). Moreover, the onset of clonic seizure in the animals treated with opipramol plus caffeine was not significantly different from the opipramol- or caffeine-treated groups (table 1). All animals, including the diazepam treated group, experienced clonic seizure after using PTZ.

Effects of Different Treatments on the PTZ-induced Generalized Tonic-Clonic Seizure

Diazepam protected all animals against the generalized tonic-clonic seizure induced by PTZ. The latency of tonic-clonic seizure was significantly different between various treatment groups ($X^2(7)=26.62$, $P<0.001$). Opipramol at a dose of 20 mg/kg and opipramol combined with ketamine or caffeine increased the latency for the onset of tonic-clonic seizure compared with the control group (table 2). However, opipramol at doses of 10 and 50 mg/kg, ketamine and caffeine treatment had no significant effect on the onset of PTZ-induced tonic-clonic seizure in mice (table 2). Diazepam decreased seizure intensity (the degree 2 compared with the degree 6 of control), but opipramol (in three doses), ketamine and caffeine had no effect on the seizure intensity in mice.

Effects of Different Treatments on the PTZ-Induced Death

Diazepam protected all animals against PTZ-induced death. Moreover, the highest level of protection against PTZ-induced death was related to the opipramol (50 mg/kg) (25%) and opipramol (10 mg/kg) or ketamine (50 mg/kg) (12.5%). However, these protections were not significantly different compared with the control groups (opipramol (50 mg/kg): $X^2=2.29$, $P=0.47$; opipramol (10 mg/kg): $X^2=1.07$, $P=1.00$; ketamine: $X^2=1.07$, $P=1.00$). Our study showed that the latency for the death of animals challenged with PTZ was significantly different between treatment groups ($X^2(7)=38.28$, $P<0.001$). Opipramol at doses of 20 and 50 mg/kg increased the latency of death of animals challenged with PTZ compared with the control group (table 3). In addition, ketamine and opipramol combined with ketamine lengthened the latency of death after using PTZ compared with the control group (table 3). However, other treatments including opipramol 10 mg/kg, caffeine, opipramol plus caffeine had no effect on the latency of death after using PTZ (table 3).

Discussion

It has been proposed that sigma receptors may be a potential target for various neuropsychiatric

Table 1: The effects of different treatments on the onset of clonic seizure induced by the pentylenetetrazole

Treatment	Onset of clonic seizure (median (IQR 25))	Statistics (Dunn's test)	P value
Opipramol (10 mg/kg)	53.00 (45.50)	14.38†	1.000
Opipramol (20 mg/kg)	77.50 (70.50)	35.94†	0.021*
Opipramol (50 mg/kg)	73.00 (70.25)	31.56†	0.093
Ketamine (50 mg/kg)	45.00 (42.50)	2.81†	1.000
Caffeine (200 mg/kg)	58.00 (56.25)	19.19†	1.000
Opipramol (20 mg/kg)+ketamine (50 mg/kg)	73.50 (65.50)	27.44† 8.50‡ –24.62§	0.313 1.000 0.670
Opipramol (20 mg/kg)+caffeine (200 mg/kg)	77.50 (67.50)	33.69† 2.25‡ –14.50#	0.046* 1.000 1.000
Diazepam (5 mg/kg)	250.00 (250.00)	56.63†	<0.001*
Vehicle	52.00 (36.00)	-	-

Drugs were administered interaperitoneally 30 minutes before the injection of pentylenetetrazole. Data presented as median and IQR 25 (inter quartile range 25) and analyzed using Kruskal-Wallis test followed by the Dunn's test. Each group consisted of 8 animals. *P<0.05; †Compared with control; ‡Compared with opipramol (20 mg/kg); §Compared with ketamine; #Compared with caffeine

Table 2: The effects of different treatments on the onset of tonic-clonic seizure induced by pentylenetetrazole

Treatment	Onset of tonic-clonic seizure (median (IQR 25))	Statistics (Dunn's test)	P value
Opipramol (10 mg/kg)	102.500 (63.25)	28.31†	0.084
Opipramol (20 mg/kg)	103.50 (71.25)	30.12†	0.0.43*
Opipramol (50 mg/kg)	88.00 (80.00)	24.19†	0.340
Ketamine (50 mg/kg)	83.00 (63.50)	25.25†	0.240
Caffeine (200 mg/kg)	104.50 (76.25)	25.44†	0.220
Opipramol (20 mg/kg)+ketamine (50 mg/kg)	145.00 (122.50)	41.56† –11.44‡ –16.31§	<0.001 1.000 1.000
Opipramol (20 mg/kg)+caffeine (200 mg/kg)	127.50 (120.00)	40.62† –10.50‡ –15.19#	<0.001 1.000 1.000
Vehicle	60.00 (48.00)	-	-

Drugs were administered interaperitoneally 30 minutes before the injection of pentylenetetrazole. Data presented as median and IQR 25 (inter quartile range 25) and analyzed using Kruskal-Wallis test followed by the Dunn's test. Each group consisted of 8 animals. *P<0.05; †Compared with control; ‡Compared with opipramol (20 mg/kg); §Compared with ketamine; #Compared with caffeine

Table 3: The effects of different treatments on the time of death of animals challenged with pentylenetetrazole

Treatment	Death time (median (IQR 25))	Statistics (Dunn's test)	P value
Opipramol (10 mg/kg)	220.00 (122.00)	20.11†	0.940
Opipramol (20 mg/kg)	313.00 (244.00)	33.56†	0.004*
Opipramol (50 mg/kg)	500 (368.75)	39.17†	0.001*
Ketamine (50 mg/kg)	420.00 (270.00)	35.04†	0.004*
Caffeine (200 mg/kg)	160.00 (112.50)	13.88†	1.000
Opipramol (20 mg/kg)+ketamine (50 mg/kg)	600 (277.50)	40.38† –6.81‡ –5.34§	<0.001* 1.000 1.000
Opipramol (20 mg/kg)+caffeine (200 mg/kg)	146.00 (130.00)	12.60† 20.88‡ 1.19#	1.000 1.000 1.000
Vehicle	107.50 (100.75)		

Drugs were administered interaperitoneally 30 minutes before the injection of pentylenetetrazole. Data presented as median and IQR 25 (inter quartile range 25) and analyzed using Kruskal-Wallis test followed by the Dunn's test. Each group consisted of 8 animals. *P<0.05; †Compared with control; ‡Compared with opipramol (20 mg/kg); §Compared with ketamine; #Compared with caffeine

disorders like epilepsy.[25] Our study showed that opipramol, a sigma receptor agonist, increased the latency for the PTZ-induced clonic and tonic-clonic seizures in mice. The exact mechanism of action of opipramol in the PTZ-induced seizure is not completely clear. However, the activation of sigma receptors may be the most probable mechanism responsible for the anti-epileptic effect of this agent. In accord with our study, Kim and his colleagues showed that carbetapentane, a sigma-1 receptor modulator, blocks the status epilepticus seizure-induced by kainic acid.[15] Moreover, it has been shown that sigma receptor agonists with low affinity for the NMDA receptors produce anticonvulsive effects in the maximal electroshock seizure in mice.[16] In addition, Guo et al. demonstrated that allosteric modulators of the sigma receptors attenuate seizures induced by different proconvulsants.[14] Therefore, our study supports previous reports about the anticonvulsant effects of sigma receptor modulators and added the anticonvulsant effect of a drug that is used clinically.

Opipramol increased the latency of death after using PTZ while had no effect on the seizure intensity in mice. In agreement with our study, it has been shown that SKF83959, a selective sigma-1 receptor modulator, protected animals from PTZ-induced mortality and had no effect on the seizure incidence after using PTZ.[14] In contrast, diazepam protected all animals from the PTZ-induced tonic-clonic seizure and death. Thus, inhibition of $GABA_A$ receptor rather than sigma receptor may be responsible for the death of animals after using high doses of PTZ.

Ketamine is an intravenous dissociative anesthetic, which is a non-competitive antagonist of the NMDA receptors.[26] Our study showed that ketamine had no effect on the onset of clonic and tonic-clonic seizure while increased the latency for the death of animals challenged with PTZ. There are some controversies in the literature about the antiepileptic effects of ketamine. Some reports have shown that ketamine produces antiepileptic effects in animal models[27,28] and in patients with epilepsy.[29] Moreover, our previous study showed that guaifenesin, a drug with antagonistic activity on the NMDA receptors, produces anticonvulsant effect in the PTZ-induced seizure.[30] In contrast, the results of some studies imply that ketamine may precipitate seizure in some brain regions of patients with epilepsy.[31]

Our study showed that caffeine alone or combined with opipramol had no significant effect on the PTZ-induced seizures. Again, there are some inconsistencies about the effects of caffeine on the seizure. There are some reports about the proconvulsant effects of caffeine in animals and patients with epilepsy.[32,33] In contrast, it has been shown that caffeine reduced susceptibility to the seizures induced by the NMDA agonists.[32] These discrepancies may be related to the species and doses of caffeine that was administered in different studies.

The present study showed that animals treated with opipramol combined with ketamine or caffeine had a higher onset of clonic and tonic-clonic seizures induced by PTZ compared with the control groups. According to our experiment, the effect of the combination of opipramol and ketamine is mainly related to opipramol. This is not surprising since, in our study, ketamine or caffeine alone had no effect on the clonic seizure. Therefore, it is possible to assume that ketamine or caffeine had no interaction with sigma receptors in PTZ-induced clonic seizure.

The main limitation of this study was the lack of selective agonist and antagonist for the investigation of opipramol mechanisms of action. Moreover, we evaluated drug effects in the PTZ model and it is necessary to investigate opipramol effects in other seizure models such as maximal electroshock seizure or kainic acid-induced seizures. Therefore, we suggest that in the future studies, it is better to use selective agonist and antagonist to explore the exact mechanism of action of opipramol and evaluate drug effect in other models of seizure.

Conclusion

Taken together, opipramol, a sigma receptor agonist, attenuated the seizures induced by the PTZ. The present study provides further support for the antiepileptic effects of sigma receptor agonists. Ketamine, an NMDA receptor antagonist, and caffeine had no interaction with the anticonvulsant activity of opipramol. The exact mechanism responsible for the antiepileptic effect of opipramol is not completely clear. However, modulation of sigma receptors may be the most probable mechanism for the opipramol antiepileptic effects.

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Conflict of Interest: None declared.

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