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Interaction of sigma-1 receptor modulators with seizure development in pentylenetetrazole-induced kindled mice

Masoumeh Emamghoreishi[a,b,c], Marzieh Shahpari[c], Mojtaba Keshavarz[d,*]

[a] Research Center for Psychiatry and Behavior Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
[b] Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[c] Department of Neuroscience, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran
[d] Shiraz Neuroscience Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

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ABSTRACT

This study aimed to investigate the effects of sigma receptor modulators, opipramol and BD-1063, on epileptogenesis in pentylenetetrazole (PTZ)-kindling model of epilepsy. Mice (n = 6/group) were received PTZ (30 mg/kg), PTZ plus opipramol (5 or 10 mg/kg), PTZ plus opipramol (5 mg/kg) plus BD-1063 (5 mg/kg, a selective sigma-1 receptor antagonist), and PTZ plus BD-1063 on alternate days for 15 days. Opipramol (5 and 10 mg/kg) + PTZ groups became fully kindled and had higher seizure scores compared to the PTZ group. In contrast, the PTZ plus BD-1063 and the PTZ plus opipramol (5 mg/kg) plus BD-1063 group did not show full kindling. These findings indicate that opipramol has a pro-convulsant effect, which is possibly mediated through activation of sigma-1 receptors.

1. Introduction

Antidepressants may produce anti- or pro-convulsant effects depending on their used doses (Dailey and Naritoku, 1996). Several studies have shown that lowering the seizure threshold may be the potential adverse effect of antidepressants (Montgomery, 2005). However, the underlying mechanism of antidepressant-induced seizure is unknown. Therefore, understanding the mechanism of antidepressants-induced epileptogenesis may help to estimate the seizure risk and to design safer drugs. Opipramol is an antidepressant, anti-anxiety, and hypnotic agent, which is used mainly in Europe (Krysta et al., 2015; Möller et al., 2001; Müller et al., 2004). There are limited data about the anti- or pro-convulsant effects of opipramol. The findings of our previous study (Keshavarz and Yekzaman, 2018) indicated that opipramol exerted beneficial effects on seizure threshold induced by a high dose of pentylenetetrazole (PTZ). However, a high dose of PTZ is mainly used to screen the possible anticonvulsant effects and is not a reliable method per se to detect anticonvulsant drugs (Kandratavicius et al., 2014). Therefore, the anticonvulsant effects of opipramol should be further confirmed in other models of epilepsy.

The chemical structure of the opipramol is similar to tricyclic antidepressants. However, opipramol is a sigma-1 and sigma-2 receptor agonist (Möller et al., 2001). The sigma-1 receptors are intracellular chaperone proteins localized in the endoplasmic reticulum (ER) membrane, mitochondria-associated endoplasmic reticulum (ER) membrane and nucleoplasmic membrane (Hayashi and Su, 2007; Su et al., 2016). Sigma-1 receptors interact with distinct receptors modulating various intracellular signal transduction systems (Guo et al., 2015; Hayashi and Su, 2007). Nevertheless, the role of sigma-1 receptors in the pathophysiology of epilepsy is elusive. There are conflicting reports regarding the effects of sigma-1 receptor modulators on epilepsy and both sigma receptor agonists and antagonists have exerted anticonvulsant activity (Vavers et al., 2017). Therefore, the aim of this study was to investigate the effects of sigma receptor modulators, opipramol and BD-1063, on epileptogenesis in PTZ-induced kindling in mice in order to clarify the contribution of sigma-1 receptors to anti- or pro-convulsant activity.

2. Methods

2.1. The experimental procedure of PTZ-induced kindling

Chemical kindling was performed according to the method described by Dhir (Dhir, 2012). Mice were intraperitoneally received PTZ (30 mg/kg, a subconvulsive dose), opipramol (5 and 10 mg/kg), and BD-1063 (5 mg/kg, a selective sigma-1 receptor antagonist) on alternate days for 15 days. BD-1063 was administered 15 and 45 min before

* Corresponding author at: Shiraz Neuroscience Research Center, Shiraz University of Medical Sciences, Chamran Hospital, Chamran Boulevard, PO Box: 7194815644, Shiraz, Iran.
E-mail address: moj.ph60@yahoo.com (M. Keshavarz).

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the injection of opipramol and PTZ, respectively. Normal saline, the vehicle for all agents, was used as the control. Immediately after PTZ injection, animal behaviors were observed for 30 min and scored using Dhir (2012) scoring method as follows:

- 0: No observed seizure activity
- 1: Myoclonic jerk (MJ)
- 3: 1 score for myoclonic jerk + 2 scores for Straub's tail
- 6: 1 score for myoclonic jerk + 2 scores for Straub's tail + 3 scores for clonus (full kindling)

The endpoint (i.e. score 6) showed full kindling of animals.

2.2. Study groups

The ethical committee of the Shiraz University of Medical Sciences approved this study. All procedures were performed according to the National Institutes of Health guide for the care and use of laboratory animals. Male BALB/c mice (2–3 months), weighing 20–40 g, were used. The animals were obtained from the Center of Comparative and Experimental Medicine, Shiraz, Iran. The ARRIVE guidelines were followed for the experimental animals. The mice had free access to a standard diet (Behparvar Co., Tehran, Iran) and water. The animals were maintained in the plexiglass cages (5/cage) in a room with controlled temperature (22 ± 2 °C) and light cycles (12:12 light: dark). Thirty-six mice were randomly allocated into six groups consisted of vehicle + vehicle (control group), vehicle + PTZ, opipramol (5 mg/kg) + vehicle + PTZ, opipramol (10 mg/kg) + vehicle + PTZ, vehicle + BD-1063 (5 mg/kg) + PTZ, opipramol (5 mg/kg) + BD-1063 (5 mg/kg) + PTZ.

2.3. Statistical analysis

The SPSS software (version 23) was used for statistical analysis. Kruskal-Wallis test followed by the Dunn's test was used for data analysis. The Chi-square test was used to compare the occurrence of each seizure behavior between groups. $P < 0.05$ was considered as statistically significant level.

3. Results

Administration of PTZ (30 mg/kg) for 15 days produced only partial kindling in mice. The concurrent injection of opipramol (5 and 10 mg/kg) and PTZ (30 mg/kg) caused full kindling in mice. Administration of BD-1063 before opipramol (5 mg/kg) and PTZ (30 mg/kg) prevented the development of full kindling in mice. The animals in PTZ + opipramol (5 mg/kg) and PTZ + opipramol (10 mg/kg) groups became fully kindled after 9 and 11 days, respectively. However, none of the animals in other groups reached full kindling within the 15 days study period.

There were significant differences in means of seizure scores between studied groups on days 3 ($\chi^2(5) = 18.107$, $p = 0.001$), 5 ($\chi^2(5) = 27.470$, $p < 0.001$), 7 ($\chi^2(5) = 25.398$, $p < 0.001$), 9 ($\chi^2(5) = 25.134$, $p < 0.001$), 11($\chi^2(5) = 26.207$, $p < 0.001$), 13($\chi^2(5) = 24.297$, $p < 0.001$), and 15 ($\chi^2(5) = 24.421$, $p < 0.001$) (Fig. 1). The post-hoc analysis showed that the means of seizure scores were significantly lower in the PTZ group on days 3 ($p = 0.005$), 5 ($p = 0.020$), 7 ($p = 0.017$), 9 ($p = 0.007$), 11 ($p = 0.10$), 13 ($p = 0.036$), and 15 ($p = 0.032$) in comparison to the PTZ + opipramol (5 mg/kg) group. Moreover, the means of seizure scores were significantly higher in the PTZ + opipramol (10 mg/kg) group on days 5 ($p = 0.003$), 7 ($p = 0.017$), 9 ($p = 0.007$), 11 ($p = 0.010$), 13 ($p = 0.036$), and 15 ($p = 0.032$) as compared with the PTZ group. In addition, PTZ + opipramol (5 mg/kg) group showed significantly higher seizure scores on days 1 ($p = 0.005$), 3 ($p = 0.003$), 5 ($p = 0.003$), 7 ($p = 0.017$), 9 ($p = 0.007$), 11 ($p = 0.032$), 13 ($p = 0.036$), and 15 ($p = 0.032$) than those of the PTZ + opipramol (5 mg/kg) + BD-1063 (5 mg/kg) group. However, there were no significant differences in means of seizure scores between PTZ + BD-1063 and PTZ groups ($p > 0.05$).

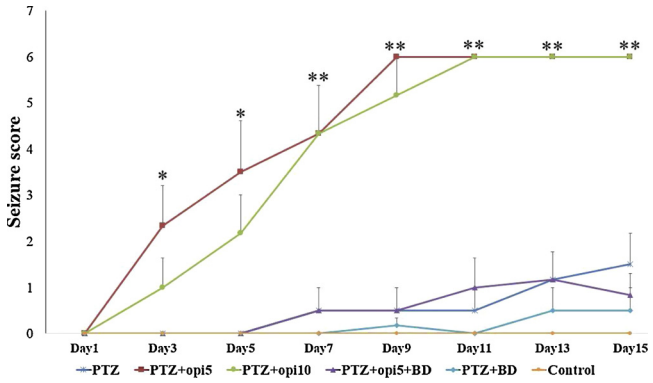

Fig. 1. Data are mean + SEM of seizure scores at different days for animals treated with pentylenetetrazole (PTZ, 30 mg/kg), opipramol (opi, 5 and 10 mg/kg), and BD-1063 (5 mg/kg) for 15 days (N = 6/ group). * and ** indicate significant values at the 0.05 and 0.001 levels, respectively, between the studied groups.

The findings also showed that there were significant differences in the occurrence of myoclonus jerk ($\chi^2(5) = 19.800$, $p = 0.001$), Straub's tail ($\chi^2(5) = 14.743$, $p = 0.012$), and generalized tonic-clonic seizure ($\chi^2(5) = 28.987$, $p < 0.001$) among the studied groups (Table 1). The occurrence of myoclonic jerk and tonic-clonic seizures were significantly lower in the PTZ + opipramol (5 mg/kg) + BD-1063 group in comparison to the PTZ + opipramol (5 mg/kg) group (Table 1). In addition, the occurrence of myoclonic jerk and Straub's tail were significantly lower in the PTZ + BD-1063 group as compared to the PTZ group (Table 1).

4. Discussion

The findings of this study showed that opipramol, a sigma receptor modulator, exerted pro-convulsant activity and decreased the threshold for seizure development in PTZ-kindling model of epilepsy. There is no controlled clinical trial about the risk of seizure occurrence following the use of opipramol. There is only a case report in the literature describing the appearance of seizure behavior in a patient who used a high dose of opipramol (Sogut et al., 2012). Our findings also showed that BD-1063, a selective antagonist of sigma-1 receptors, inhibited the pro-convulsant effects of opipramol. Previous studies about the effects of sigma receptors on seizure threshold have produced inconsistent results. Vavers et al. (2017) showed that the acute administration of PRE-084, a selective sigma receptor agonist, had no effect on seizure threshold in PTZ-infused mice. However, they have reported that NE100, a sigma receptor antagonist, raised the seizure threshold in

Table 1
Data are the numbers (percentages) of animals showed myoclonic (MJ), Straub's tail (ST), and tonic-clonic (TC) seizures in each studied group (N = 6/group) including PTZ (pentylenetetrazole, 30 mg/kg); PTZ + opi5 (opipramole 5 mg/kg); PTZ + opi10 (opipramole 10mg:kg); PTZ + opi5 + BD-1063 (5 mg/kg), PTZ + BD-1063, and control (normal saline).

Treatment	MJ (%)	ST (%)	TC (%)
PTZ	3 (50)	6 (100)	1 (16.7)
PTZ + Opi5	6 (100)	2 (33.3)	6 (100)
PTZ + Opi10	5 (83.3)	3 (50)	6 (100)
PTZ + Opi5 + BD-1063	1 (16.7)	3 (50)	0
PTZ + BD-1063	1 (16.7)	1 (16.7)	1 (16.7)
Control	0	0	0

PTZ-infused mice (Vavers et al., 2017). On the other hand, Shin et al. (2005) reported that some sigma-1 receptor agonists such as dextromethorphan and dimemorfan have diminished kainic acid-induced seizure in rats. Our previous study also showed that opipramol increased the latency time to seizures in an acute model of PTZ-induced seizure in mice (Keshavarz and Yekzaman, 2018). The discrepancy between the findings of this study with the previous one may be attributed to the use of different models of epilepsy i.e. kindling model vs the acute model. It is noteworthy that the kindling model is a more accurate method to evaluate the pro- or anticonvulsant effects of drugs.

The present study showed that opipramol, a sigma-1 receptor agonist, facilitated the development of kindling seizure development. In contrast, a selective sigma-1 receptor antagonist has reversed the proconvulsant effect of opipramol. The mechanism underlying the proconvulsant activity of opipramol is currently unknown. However, sigma-1 receptor agonists activate neuronal firing in the hippocampus by potentiating NMDA receptors (Monnet et al., 1990). In addition, upregulation of NMDA receptors in the hippocampus and cortex of PTZ-induced kindled animals has been reported (Ekonomou and Angelatou, 1999). Therefore, it can be proposed that the chronic administration of a sigma-1 receptor agonist may increase the risk of epileptogenesis in predisposed animals possibly through its enhancing effects on NMDA receptors. On the other hand, the affinity of opipramol for other receptors, such as dopamine and serotonin, might be contributed to its pro-convulsant activity as well. This notion should be addressed in future studies.

5. Conclusion

The sub-chronic administration of opipramol facilitated epileptogenesis in predisposed animals while a sigma receptor antagonist reversed this effect. This suggests that activation of sigma-1 receptors may be involved in the pro-convulsant effects of opipramol.

Conflict of interest

None declared

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