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Synthesis and Study of Anti-convulsive Effect of 1-[1-(4-Methylphenyl) (Cyclohexyl)] 4-piperidinol as a New Derivative of Phencyclidine by PTZ-Induced Kindling Model in Male Mice

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A B S T R A C T

Background and Objective: Regarding the prevalence of epilepsy in human society and with respect to insufficiency of usual treatments, new strategies and methods for medical treatment of epileptic patients are necessary. As NMDA receptor antagonists are the most prominent anti-epileptic drugs, in the present study, we synthesized and investigated anti-epileptic effect of a new piperidine derivates 1-[1-(4-Methylphenyl) (Cyclohexyl)] 4- piperidinol as a new NMDA receptor antagonist in chemical kindling model.

Materials and Methods: Sixty male mice (NMRI), weighing 25-30 g, were selected and randomly divided into 5 groups (n=12 in each group). 1: PTZ 2**:** 1-[1-(Phencyclohexyl) piperidine, PCP)] 3: 1-[(1-3-Methoxy phenyl tetralyl) piperidine)] 4: 1-[1-(4-Methylphenyl) (Cyclohexyl)] 4- piperidinol and 5: valproic acid (positive control). Chemical kindling was induced by PTZ (35 mg/kg, i.p) injection, 11 times on alternate days (22 days). In final injection (challenge dose) at 24th day, PTZ were applied with 75 mg/kg to the animals. Thirty minutes after PTZ injection, the animals were followed for convulsion scores (0-5).

Results: Data analysis showed that administration of 1-[1-(4-Methylphenyl) (Cyclohexyl)] 4- piperidinol has a prominent anti-convulsion effect versus PCP, especially in reduction of phase 2 duration. Meanwhile, this compound had a marked anti-epileptic effect in challenge dose.

Conclusion: The results suggest that administration of the new piperidine derivate, 1-[1-(4-Methylphenyl) (Cyclohexyl)] 4- piperidinol could yield a prominent anti-convulsion effect in grand mal epilepsy. Regarding changes of its conformation as a non-competitive antagonist, it may block the NMDA receptors more powerfully than other piperidine derivates.

Key Words:
Convulsion
Piperidine derivates
Chemical kindling
PTZ

1. Introduction

Phencyclidine (1-(1-phenylcyclo-hexyl) piperidine, CAS 956-90-1, PCP, **I**) is a semi-rigid molecule containing a cyclohexane ring with attached aromatic and piperidine rings (Scheme 1). PCP and its analogues are highly potent and widely abused psychotomimetic drugs which influence the central nervous system and display analgesic, stimulant, depressant and hallucinogenic effects because of specific binding sites in the brain (1).

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PCP binds to the N-methyl-D-asparate (NMDA) receptor complex and blocks NMDA-mediated gating of the calcium conductance channel (2). The analgesic effect of ketamine (2-O-chlorophenyl-2-methylaminocyclohexan, CAS 1867-66-9, II, Scheme 1), another PCP analogue, was first described by Domino, et al in 1965 (3). Ketamine is a low-affinity, use-dependent, noncompetitive antagonist of NMDA receptors (4-6).

Recently, many analogues of phencyclidine have been synthesized (7-18) and their pharmacological activities have been studied. As part of our efforts to reach selective, non-competitive antagonists at the PCP binding site on NMDA receptor complex, we have prepared 1-[1-(4-Methylphenyl) (Cyclohexyl)] 4-piperidinol , (PCP-OCH_3-tetralyl, III, Scheme 1), as an analogue of PCP with a methoxy group on the aromatic ring (*m*-position) and a phenyl group with cyclohexane ring (a conjugated cyclic ketone, 1-tetralone) to examine its anti-convulsion effects on mice by PTZ – induced kindling model. The results have been compared to PCP and valproic acid. It was anticipated that incorporation of methoxy group on the aromatic ring of the molecule will produce pronounced effects on electron distribution and dipole moments because of the high electron donating character of this group (7). Also, incorporation of an extra aromatic and flat phenyl group with cyclohexane ring (a conjugated cyclic ketone, 1-tetralone) was anticipated to decrease the conversion of conformation isomers of the drug (19-21).

2. Materials and Methods

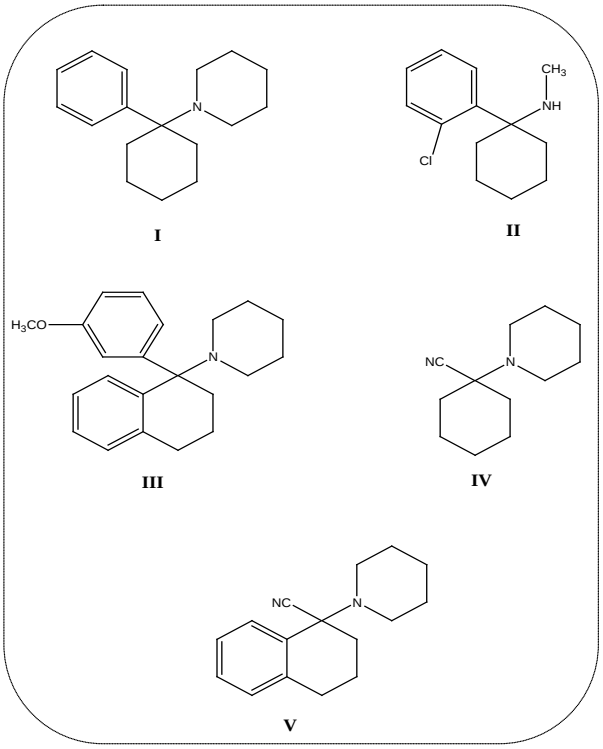
2.1. Experimental

1-Tetralone [1, 2, 3, 4-Tetrahydro-1-naphthalenone], Cyclohexanone, Piperidine, Bromo benzene, Magnesium turning, Diethyl ether, 3-bromo anizole, and all other chemicals, were purchase from Merck chemical Co. (Darmstadt, Germany). Melting points (uncorrected) were determined using a digital Electrothermal melting point apparatus (model 9100, Electrothermal Engineering Ltd., Essex, UK). ^1H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz (model AMX, Karlsruhe, Germany) spectrometer (internal reference: TMS). IR spectra were recorded on a Thermo Nicolet FT-IR (model Nexus-870, Nicolet Instrument Corp, Madison, Wisconsin, U.S.A.) spectrometer. Mass spectra were recorded on an Agilent Technologies 5973, Mass Selective Detector (MSD) spectrometer (Wilmington, USA). Column chromatographic separations were performed over Acros silica gel (No.7631-86-9 particle size 35-70 micrometer, Geel, Belgium). Adult male mice (Razi Institute, Tehran, Iran), weighing 22 -26 g were used for pharmacological testing.

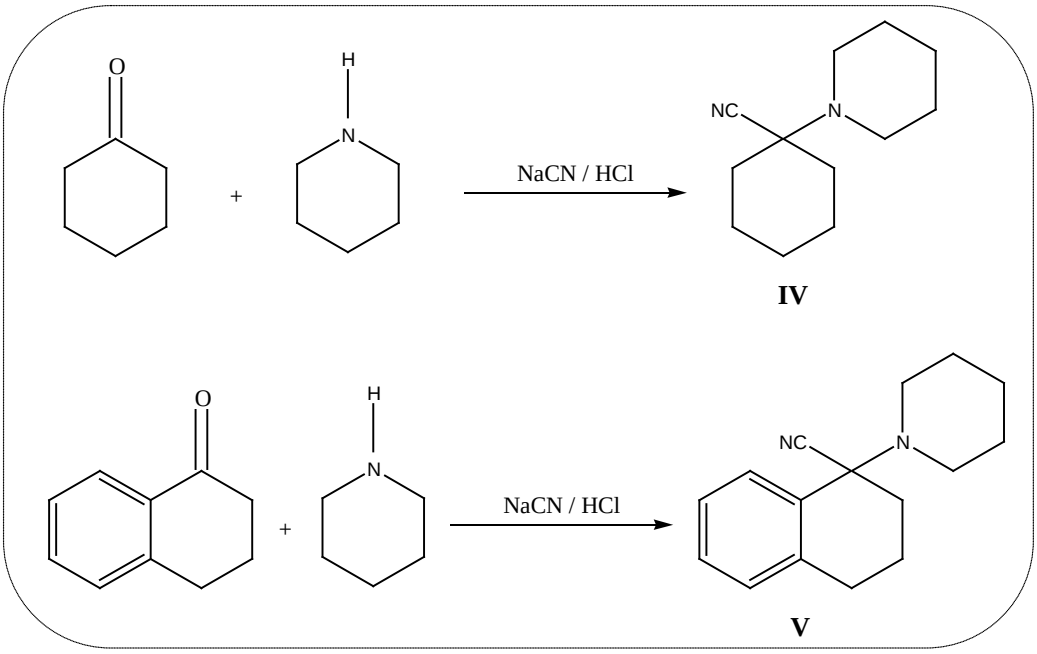
2.2. Synthesis of compounds (Scheme 1, 2)

(1-(1-phenylcyclohexyl) piperidine (PCP) I

This compound was prepared according to reported method (22) from 1-piperidinocyclohexanecarbonitrile (**IV**) and phenyl magnesium bromide. The hydrochloride salt of I was prepared using 2-propanol and HCl and was recrystallized from 2-propanol (22).



Scheme 1. Structure formulas of PCP (**I**), Ketamine (**II**), PCP-OCH_3-tetralyl (**III**) and Carbonitrile intermediates **IV** and **IV**.



Scheme 2. Synthesis of intermediates **IV** and **V**.

1-Piperidinotetralylcarbonitrile V

To a solution of containing 0.582 g (0.0068 mol) of piperidine in 0.253 g HCl (37%) and 1.36 g cold water, 1 g (0.0068 mol) 1, 2, 3, 4-tetrahydro-1-naphtalenone (1-tetralone) was added. Then, 0.465 g KCN in 1.02 ml water, 50 ml ethanol and 0.1 g tetra-n-buthylammonium bromide (0.0003 mol) were added and stirred in ambient temperature (25° C). The progress of reaction was controlled by TLC (7:3 ethyl acetate/n-Hexane). After one week no additional progress was seen, so the reaction was extracted with Chloroform (75 ml, 3 times). Then organic layer was separated, dried and concentrated. The oily residue was obtained, which was passed through a silica gel column using ethyl acetate-hexane (7:3) as the eluent to afford 1.13 g of **V** (69 % yield).

IR (KBr): 3066, 2941, 2560, 1454, 1436, 1324, 1287, 1225, 764 cm^{-1}.

^{1}H N.M.R. ($CDCl_3$) (p.p.m.): *1.5-2.85 (16H, m), 6.93-7.01 (4H, m).*

^{13}C N.M.R. (CDCl3) (p.p.m.): *25.4, 26.2, 26.8, 31, 37.9, 46.7, 52.7, 117.7, 125.5, 128.1, 139.2.*

MS: m/z (regulatory intensity): 240 $[M]^+$ (76), 241 $[M+ H]^+$(15).

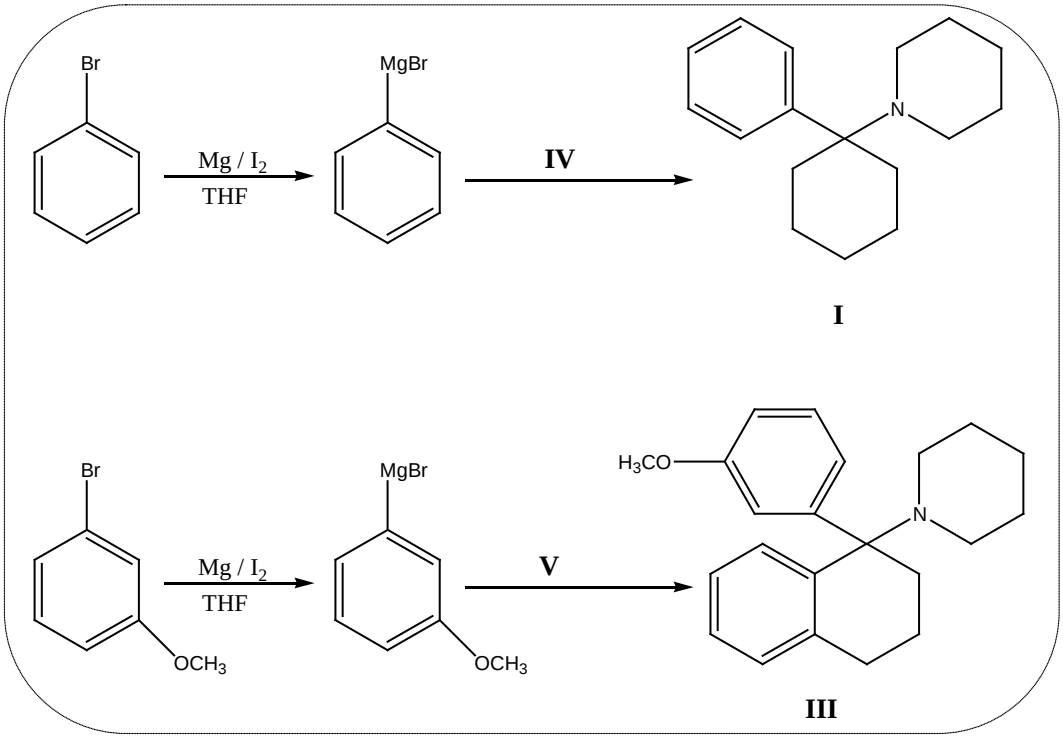
1-[1-(4-Methylphenyl) (Cyclohexyl)] 4-piperidinol III

A solution containing 4 g (0.016 mol) of nitrile compound (**V**) in 10 ml of dry THF was added to a refluxing solution of (3-methoxylphenyl) magnesium bromide (Grignard reagent) (prepared from 24.77 g 3-bromoanisole and 3.075 g of Mg in 17 ml of dry ether), refluxed for 5 additional h in 65-67 °C, left overnight at ambient temperature (25 °C) and then poured into ice-NH_4Cl. The organic layer was separated and washed with water and the base was neutralized with 10% H_2SO_4, washed with 20% NaOH, re-extracted with n-Hexane, dried and concentrated. The oily residue was obtained, which was passed through a silica gel column using ethyl acetate-hexane (7:3) as the eluent to afford 2.28 g of **III** (42 % yield).

The hydrochloride salt of **III** was prepared using 2-propanol and HCl and was recrystallized from 2-propanol.

IR (KBr): 3066, 2941, 1602, 1483, 1454, 1436, 1324, 1287, 1225, 764 cm^{-1}.

^{1}H N.M.R. ($CDCl_3$) (p.p.m.): *1.5-2.85 (16H, m), 3.73 (3H, s), 6.59-7.1 (8H, m).*



Scheme 3. Synthesis of compounds **I** and **III**.

^{13}C N.M.R. (CDCl3) (p.p.m.): *26.2, 27.5, 31.8, 44.8, 47.4, 56, 63, 111.6, 114, 120.2, 120.7, 125.8, 126.2, 128.8, 130, 139.3, 142.8, 144, 162.5.*

MS: m/z (regulatory intensity): 321 $[M]^+$ (100), 322 $[M+ H]^+$(7).

2.3. Experimental procedures

Animals

In this experimental research, a total of 60 mices (NMRI) weighing 22-26 g (Razi Institue, Tehran, Iran) were randomly divided into six groups including; 1-control, 2- PTZ, 3-positive control (PTZ and valproate 100 mg /kg; i.p. as an anti-convulsant drug), 4, 5 PCP and its new compound methoxy PCP, respectively. Ten mice were housed in each cage at a temperature of 21±2ºC and 12 h light-dark cycle. The mice had free access to standard food and tap water *ad libitum*. The experimental protocol was approved by the Ethic Committee of University.

2.4. Kindling

All animals but control group (group 1) were kindled by a total of 11 periodic injections of PTZ (35 mg/kg; i.p.). Each administration was carried out every second day for a period of 22 days. The challenge dose of 75 mg/kg PTZ was injected in kindled mice on day 24 (test day). The challenge dose injection of PTZ produced convulsions (clonic and tonic) with lethality. All kindled mice were tested for PTZ challenge dose (75 mg/kg)-induced seizures and status. However, the exhibited phases of seizure (0-6) were observed and categorized using following scale (18) for 30 minutes after PTZ injection. The scale introduces six phases as follows:

0: no response

1: ear and facial twitching

2: convulsive waves axially through the body

3: myoclonic body jerks

4: generalized clonic convulsions turn over into side position

5: generalized convulsions with tonic extension episode and status epilepticus

6: mortality.

2.5. Statistical analysis

Data were expressed as means ± S.E.M. Statistical analyses was carried out using repeated measurement of one way analysis of variance (ANOVA) followed by post-hoc Tukey test and p values less than 0.05 were considered as significant differences.

3. Results

3.1. Chemistry

Phencyclidine (I), and 1-[1-(3-methylphenyl) (tetralyl) piperidine (III) were synthesized by reaction of substituted Grignard reagents and carbonitrile compounds (IV, V). To obtain higher electron distribution and dipole moment properties, a methyl group was substituted on the aromatic ring of the molecule (III). Known procedures were applied for the synthesis of compounds I and IV with the appropriate modifications described previously (19, 22).

Bromobenzene and its *m*-methoxy (II) derivative were reacted with magnesium to form Grignard reagents, which were then reacted with appropriate piperidinocyclohexanecarbonitrile (IV) and piperidinotetralylcarbonitrile (V). Reaction between the Grignard reagents and the carbonitriles were slow and incomplete. So to overcome this problem, molar ratio of Grignard reagents to carbonitriles were increased (19, 31).

Spectroscopic data (IR, ^{1}H and ^{13}C NMR, Mass) confirmed the structure of compounds III and V. The melting points of known compounds could also confirm their identity. The purity of each compound was checked by TLC using ethyl acetate/n-hexane as the eluent.

3.2. Pharmacology

General Consideration

Mortality, morbidity, irritability and other side effects due to drugs administration were not observed. However, comparison of the motor coordination index (was measured by Rota-rod apparatus, Harvard, UK) indicated no significant differences between control and treatment rat.

3.3. Effect of PCP_1 on the PTZ-induced kindling intensity

Statistical analysis of results (as are shown in figure 1) indicates that there are no significant differences among experimental groups in the seizure intensity till 5th injection. As it is shown in figure 1, PCP injection (5.6 mg/kg) at 9, 11 and specially 12th injection could able to reduced PTZ-induced seizure significantly ($p<0.05$). However, valproate (150 mg/kg) has reduced seizure intensity in all periods significantly ($p<0.05$). At the 12 injection (challenge dose), PCP had more significant reduction effect on seizure intensity than that of valproate ($p<0.001$).

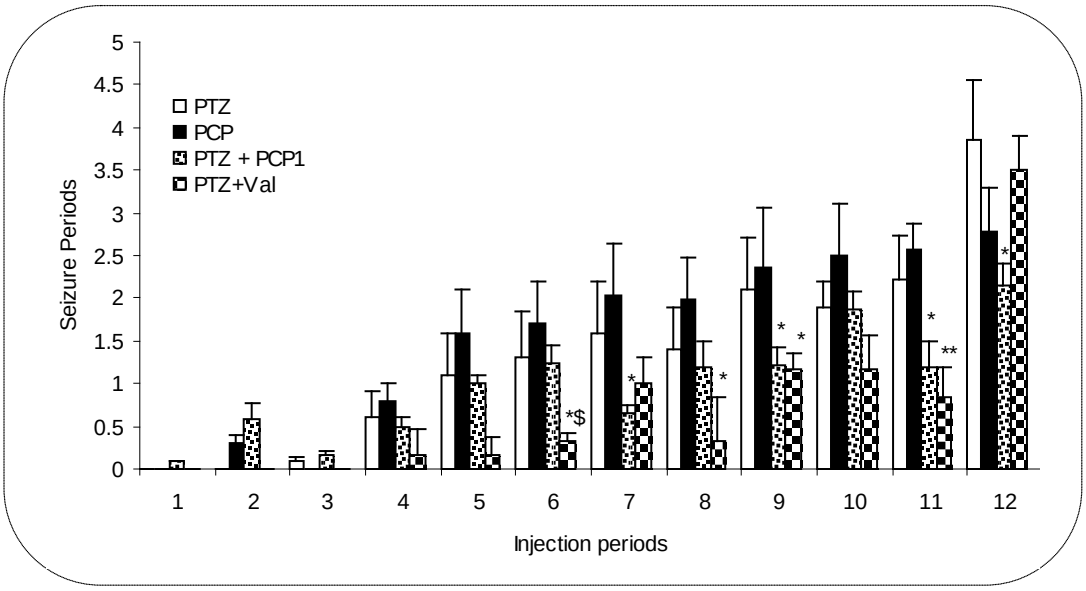


Figure 1. Effect of PCP and PCP_1 pretreatment on the PTZ-induced kindling intensity. *P < 0.05 and **P < 0.01 indicate significant differences as compared to PTZ-kindled group.

Figure 2. Effect of valproate (150 mg/kg), PCP and PCP1 on the latency of arriving to phase 5 of seizure (n=8 in each group)

Group test	Phase 5 latency time (s)	Phase 5 duration time (s)	Mortality (%)	Chimney test analysis
				% of mice showing motor impairment
PTZ	3.86 ± 0.70	4.51 ± 0.58	10.20	0
PTZ + VA	3.50 ± 0.60	3.15 ± 0.45	0	0
PCP	2.79 ± 0.85	4.15 ± 0.48	12.50	8.33
PTZ + PCP-CH3-OH	3.78 ± 0.38	3.90 ± 0.55	0	6.33

Figure 3. Effect of valproate (150 mg/kg), PCP and PCP1 on the remaining time in the phase 2 and 5. (n=8 in each group)

Group test	Phase 2 latency time (s)	Phase 2 duration time (s)	Phase 5 latency time (s)	Phase 5 duration time (s)
PTZ	4.41 ± 0.52	27.19 ± 2.19	3.33 ± 0.86	4.11 ± 0.48
PTZ + VA	3.66 ± 1.17	9.50 ± 1.55*	2.12 ± 0.60	2.15 ± 0.45*
PCP	5.81 ± 0.55	9.72 ± 0.74*	4.75 ± 0.90	4.15 ± 0.64
PTZ + PCP-CH3-OH	5.22 ± 1.35	10.75 ± 1.70*	4.02 ± 0.48	4.11 ± 0.87

3.4. Effect of PCP1 on the PTZ-induced kindling factors

As could be seen in table 1, pretreatment of animals with PCP and PCP1 have not any significant effect on the duration time which the mice reach to phase 5 seizures. In addition, table 2 indicates that only pretreatment of mice with PCP1 and valproate 150 mg/kg are able to significantly reduce the period that mice remained in phase 2 of seizure ($p<0.05$).

4. Discussion

Electrophysiological and binding studies have revealed that when the channels are in the open or activated state various antagonists of NMDA receptors, including phencyclidine, ketamine and MK-801 primarily binds to PCP-site (9, 32). Previous studies suggest that ketamine may interact with the NMDA receptor at two potentially distinct sites: one located within channel pore and the second site associated with hydrophobic domain of the protein. The binding of the agonist to the receptor is assumed to modify the binding of Ketamine to both sites (33).

In this work, a new derivative of PCP (III) and its carbonitrile intermediate having changes in substitutions in its phenyl and cyclohexane rings was synthesized. Because of stronger analgesic effects of some our synthesized derivatives of PCP with methyl, methoxy, hydroxyl groups on phenyl and cyclohexane rings (8, 15, 19) and more electron distribution and dipole moments of methoxy group (7) and for decreasing the conversion of conformation isomers of the drug (20, 21), we synthesized and studied the analgesic effects an new analogue of PCP with two additional groups on phenyl and cyclohexane rings of the molecule (III).

Comparison of the anticonvulsant data indicated that PCP-OCH_3-tetralyl could diminish seizure phase especially in full kindling level. It showed that perhaps different mechanisms are involved in PCP and PCP-OCH3 for such different responses. However, the long lasting effect for methoxy compound as compared to PCP, could be related to its higher half life. Perhaps, the higher permeability of blood brain barrier (BBB) to PCP-OCH_3-tetralyl explain its potent anti-epileptic activity.

Nowadays, NO is known as an important neurotransmitter that in addition to various physiological duties, is also related to synaptic plasticity, neuronal excitability regulation, and epileptic activity (8). Involvement of NO in epilepsy is approved via different experiments and systemic injection of NOS (NO synthase)

inhibitors (8, 31). Controversial effects of NO on the PTZ-induced convulsant have been obtained. Oliveria and colleagues have shown that NOS inhibition in kindling model amplifies the 60 mg/kg PTZ-induced seizure intensity, but has protective effect against 80 mg/kg PTZ-induced tonic seizure (32). So, they have concluded that preconvulsant or anticonvulsant activity of NOS and NO inhibitors is dependent on the PTZ dose and the seizure model. They have attributed the protective and inhibitory effect of NOS on the high dose of PTZ to the contribution of NO in the proconvulsant effect of limbic system (32, 33). It has been reported that PTZ-induced seizure are modulated by endogenous NO production and glutamate ionotropic receptors (Itoh K and Watanabe M 2009). Using nNOS $^{-/-}$ mice (lacking nNOS gene) and nNOS (neuronal NO synthase) inhibitors, they have concluded that basic and enhanced levels, implies negative and positive modulatory effects respectively (34). PTZ daily injection via NMDA glutamate receptor activates calcium release via NMDA receptor that consequently activates calcium-calmodoline pathway to increase nNOS protein expression and NO increment in brain different area. The higher NO level is able to increase the induction of generalized epilepsy (34). NO has complex effect on the neurotransmitters, by which it could mediate the cGMP activation resulted from NMDA receptor activation but simultaneously inhibit the intracellular Ca^{2+}, NOS activation and NMDA receptor (35). It has been shown that NO is able to inhibit synaptic membrane connected L-glutamate in mouse brain in a dose dependent manner (36). It is suggested that anticonvulsant role of NO is related to an implied feedback of NO on the NMDA receptor activation via different mechanisms (8). However, NO is known as a molecule that can easily react with O_2 free radicals in the brain and reduce the oxidative stress-induced damage via deleting free radicals (24). Controversial results makes difficulties to predict pro or anti convulsant effect of NO molecule. Anyway, in the present research, the NO level is decreased in PTZ and PCP groups. This indicates that probably PCP could have suppressing effect on the seizure via NO synthesis pathway activation. Probably, the reduced level of NO in PTZ mice is resulted from free radicals production at seizure time, and its consumption due to its cleaning effect.

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