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Original article

The Effect of Phencyclidine New Derivatives on Anxiety Behaviors in Rats

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Abstract

Objective(s)
Anxiety is a common disorder which afflicts many people in any society and is often accompanied by physiological sensations such as tachycardia, chest pain, shortness of breath, insensitivity, etc. The purpose of present study was to evaluate the putative anxiolytic-like effects of phencyclidine (1-(1-phenylcyclohexyl) piperidine, CAS 956-90-1, PCP, I) and its methyl and methoxy hydroxyl derivatives (II, III) using elevated plus maze test of anxiety.

Materials and Methods
Phencyclidine as well as its methyl and methoxy hydroxyl derivatives (I, II, III) (hydrochloride, 1, 2, 5 mg/kg) were synthesized and administrated intraperitoneally (IP) on adult male Wistar rats.

Results
The results of this study demonstrated that, intraperitoneal (IP) administration of PCP analogues (I, II, III) hydrochloride (1, 2, 5 mg/kg) increases the percentage of open arm time (OAT%) and percentage of open arm entries (OAE%).

Conclusion
This study revealed that both derivatives of phencyclidine (II, III) were more effective than PCP (I) itself in modulation of anxiety behavior in rats.

Keywords: Anxiety, Methyl and methoxy hydroxyl derivatives, Phencyclidine

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Introduction

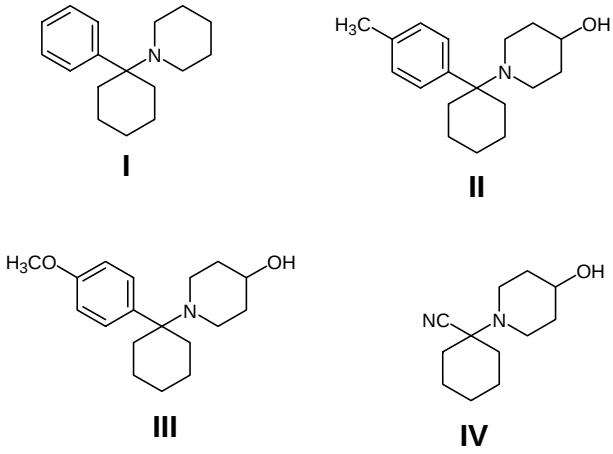
Phencyclidine (1-(1-phenylcyclohexyl) piperidine, CAS 956-90-1, PCP, I, Scheme 1) and its derivatives because of specific binding sites in the brain (1), display analgesic (1-3), stimulant (4), anticonvulsant (5) and behavioral effects (6, 7).

PCP binds to N-methyl-D-asparate (NMDA) receptor complex and blocks NMDA-mediated gating of the calcium channel conductance (8). These classified performance may have many behavioral effects in common with other phencyclidine-like drugs, including anesthetics, antinociceptives, psychomimetics, anticonvulsants, neuroprotectives and amnesic drugs related to non-competitive, "open channel blockers" of the NMDA receptor (9).

Anxiety as a model of behavioral effect of many drugs such as PCP and its analogues, morphine, amphetamine, dexfenfluramine, diazepam, etc. have been studied on laboratory animals and the findings indicated that different brain systems are involved in such behavioral effects (10-12).

Experimental evidence gathered over the last decade has established that excitatory amino acids (EA), such as glutamate, act as a neurotransmitter in the mammalian central neurvous system, and are likely to be involved in several important physiological and pathophysiological processes (13, 14). However, regarding to behavioral functions, anxiolytic effects have been reported in animal models of anxiety following administration of antagonists of the NMDA receptor. At least three types of EA receptors, named after their preferential agonists N-methyl-D-aspartate (NMDA), quisqualate and kinate have been identified. NMDA receptor antagonists have shown to be anxiolytic in animal models of anxiety (15, 16). These compounds bind to several specific sites within the NMDA-receptor complex, including the NMDA site itself, the phencyclidine site, and the strychnine-insensitive glycine site (16, 17).

In this work, methyl and methoxy hydroxyl derivatives of phencyclidine [(1-[1-(4-methylphenyl) (cyclohexyl)] 4-piperidinol, II), (1-[1-(4-methoxyphenyl) (cyclohexyl)] 4-piperidinol, III)] as NMDA receptor antagonists, were tested for food and water intake on rats and the results are compared to PCP and vehicle.



Scheme 1. Structures of PCP (I), PCP-CH_3-OH (II), PCP-OCH_3-OH (III) and Carbonitrile intermediates 1 and IV.

Material and Methods

Chemistry

Cyclohexanone, piperidine, bromo benzene, magnesium turning, diethyl ether, 4-bromo toluene, 4-bromo anisole, 4-piperidinol and all other chemicals, were purchased 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). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz (model AMX, Karlsruhe, Germany) and spectrometer (internal reference: TMS). IR spectra were recorded on a Thermo Nicolet FT-IR (model Nexus-870, Nicolet Instrument Corp, Madison, Wisconsin, USA) spectrometer. Mass spectra were recorded on an Agilent Technologies 5973, Mass Selective Detector (MSD) spectrometer (Wilmigton, USA). Column chromatographic separations were performed over Acros silica gel (No.7631-86-9 particle size 35-70 micrometer, Geel, Belgium).

Preparations (Scheme 1)

4-hydroxypiperidinocyclohexylcarbonitrile (IV)

This compound was prepared in an organic solvent according to Genestee *et al* (18), from 4-piperidinol, cyclohexanone and KCN.

1-(1-phenylcyclohexyl) piperidine (PCP) (I)
This compound was prepared according to a Maddox *et al* 1965 (19) from 1-piperidinocyclohexanecarbonitrile and phenyl magnesium bromide. The hydrochloride salt of I was prepared using 2-propanol and HCl and was recrystallized from 2-propanol.

1-[1-(4-methylphenyl) (cyclohexyl)] 4-piperidinol (MC)(II)
This compound was prepared from nitrile compound (IV) and *p*-tolyl magnesium bromide (Grignard reagent) according to a published method (20).

The hydrochloride salt of II was prepared using 2-propanol and HCl and was recrystallized from 2-propanol (20).

1-[1-(4-methoxyphenyl) (cyclohexyl)] 4-piperidinol (OMC) (III)
This compound was prepared from nitrile compound (IV) and *p*-anisol magnesium bromide (Grignard reagent) according to Ahmadi *et al* 2009 (20).

The hydrochloride salt of III was prepared using 2-propanol and HCl and was recrystallized from 2-propanol (20).

Animals
Adult male Wistar rats (Pasteur Institute, Tehran, Iran), weighing 220-270 g were housed in individual polypropylene cages under controlled temperature (25° C) and light (12 hr: 7 am to 7 pm) /dark (12 hr) cycle as well as free access to food and water. The experimental procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH) and those of the Research Council of Department of Biology, Karaj Islamic Azad University –Karaj branch.

Plus-maze
Plus-maze is a wooden and plus-shaped apparatus that was elevated to a height of 50 cm, and consists of two 50×10 cm open arms, and two 50×10×50 cm enclosed arms, each with an open roof. The maze was placed in the center of a quiet and dimly lit room. The rats' behavior was directly observed using a mirror suspended at an angle above the maze. Behavioral data was collected by a "blind" observer who quietly sat 1 m behind one of the closed arms of the maze, using a chronometer. Five min following their respective drug treatment, rats were placed individually in the center of the plus-maze, facing one of the closed arms. The observer measured 1) time spent in the open arms, 2) time spent in the closed arms, 3) number of entries into the open arms, and 4) number of entries into the closed arms during the 5 min test period. An entry was defined as all four paws in the arm. The maze was cleaned with distilled water after each test. For the purpose of analysis, open-arm activity was quantified as the duration of time that the rat spent in the open arms relative to the total duration of time spent in the other arms (open/total×100), and the number of entries into the open arms was quantified relative to the total number of entries into the remaining arms (open/total×100). The total number of arms entered, as well as the total number of closed arms entered was used as indices of general locomotor activity (21).

Drug treatments
Phencyclidine (I), 1-[1-(4-methylphenyl) (cyclohexyl)] 4-piperidinol (II) and 1-[1-(4-methoxyphenyl) (cyclohexyl)] 4-piperidinol (III) hydrochloride were dissolved in 0.2 ml saline and injected intraperitoneally (i.p.).

Experiment 1
Effect of phencyclidine (PCP) on anxiety behavior
Four groups of rats were tested with phencyclidine. First group received i.p. injection of saline (0.2 ml) and other three groups received i.p. injection of phencyclidine (1, 2 and 5 mg/kg)

Experiment 2
Effect of 1-[1-(4-methylphenyl) (cyclohexyl)] 4-piperidinol (MC) (II) on anxiety behavior
Four groups of rats were tested with phencyclidine. First group received i.p. injection of saline (0.2 ml) and other three groups recived i.p. injection of MC (1, 2 and 5 mg/kg)

Experiment 3
Effect of 1-[1-(4-methoxyphenyl) (cyclohexyl)] 4-piperidinol (III) hydrochloride (OMC) on anxiety behavior
Four groups of rats received i.p. injection of saline (0.2 ml) or OMC (1, 2 and 5 mg/kg)

Statistical analysis
Data displayed normal distribution and homogeneity of variance, therefore one-way ANOVA was used for comparison between the effects of different doses of drugs with vehicle (saline).

Results
Phencyclidine (I), 1-[1-(4-methylphenyl) (cyclohexyl)] 4-piperidinol (II) and 1-[1-(4-methoxyphenyl) (cyclohexyl)] 4-piperidinol (III) were synthesized by reaction of substituted Grignard reagents and carbonitrile compounds. These compounds (II, III) show strong hydrophilic and polarity (a hydroxyl group on the piperidine ring) and high electron donating, distribution and dipole moments (a methyl (II) or methoxy (III) group on the aromatic ring) properties. Known procedures were applied for the synthesis of all compounds I-IV with the appropriate modifications described previously (18-20).

Animal behavioral observation showed no mortality, morbidity, irritability and other side effects due to drugs administration.

The effects of phencyclidine (PCP) on anxiety behavior
Figure 1 shows the effect of IP injection of phencyclidine (1, 2 and 5 mg/rat) in the elevated plus-maze in rats. One-way ANOVA revealed that PCP increased %OAT at the dosages of 2 mg/kg ($P< 0.05$). No change in the %OAE and locomotor activity was observed.

The effects of MC on anxiety behavior
One-way ANOVA revealed that IP injection of MC (1, 2 and 5 mg/rat) increased %OAT (*$P< 0.05$, **$P< 0.01$ and ***$P< 0.001$) and %OAE (*$P< 0.05$ and **$P< 0.01$) at the doses of 1, 2 and 5 mg/rat. No significant change in the locomotor activity was observed (Figure 2).

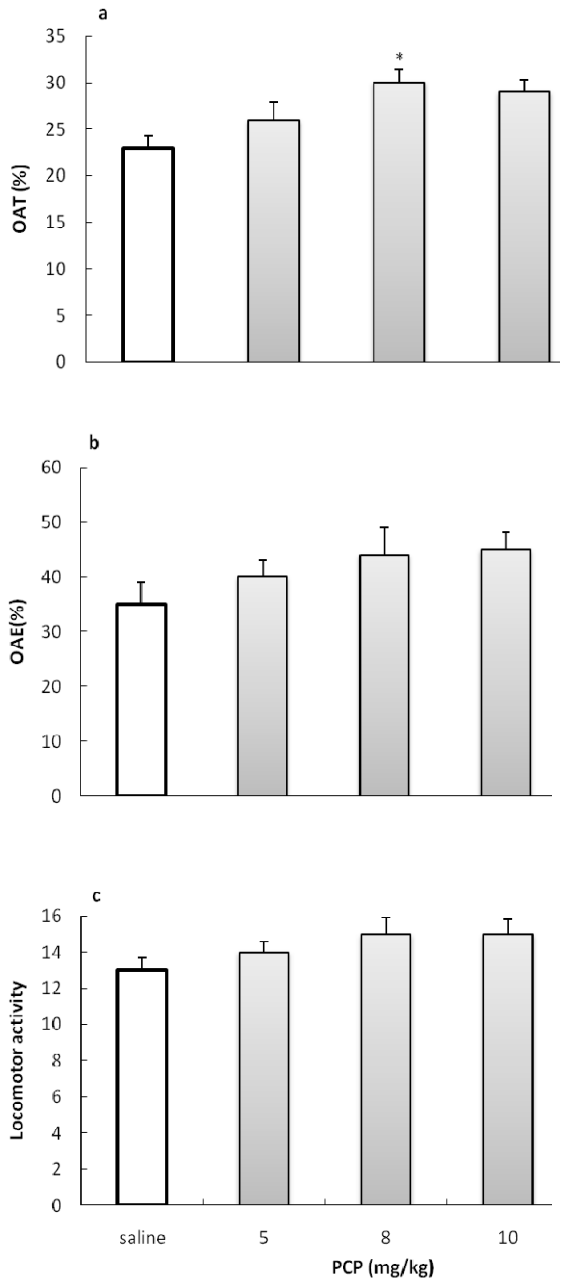


Figure 1. The effects of IP injection of PCP (1, 2 and 5 mg/kg) on %OAT (a), OAE (b) and locomotor activity (c). *$P< 0.05$ compared with saline-injected rats (Mean±SEM, n=7).

The effects of OMC on anxiety behavior
Figure 3 shows the effect of IP injection of OMC (1, 2 and 5 mg/rat) in the elevated plus-maze in rats. One-way ANOVA revealed that OMC increased %OAT (*$P< 0.05$ and **$P< 0.01$) and %OAE (*$P< 0.05$ and **$P< 0.01$) at the dosages of 1, 2 and 5 mg/kg. No change in the locomotor activity was observed.

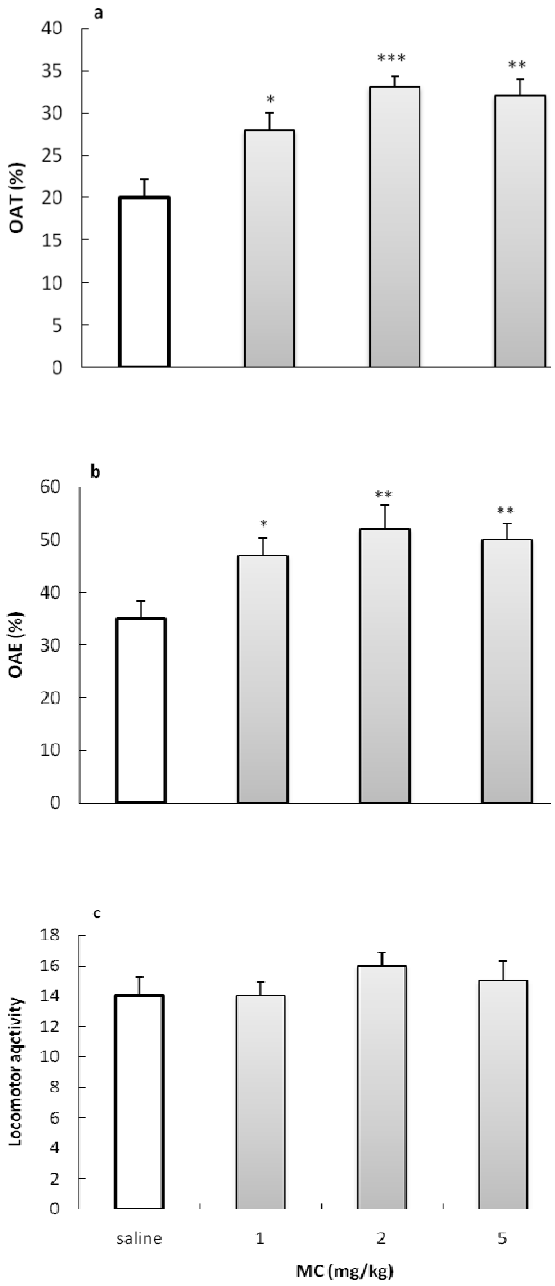


Figure 2. The effects of IP injection of MC (1, 2 and 5 mg/kg) on %OAT (a), OAE (b) and locomotor activity (c). **P*< 0.05, ***P*< 0.01 and ****P*< 0.001 compared with saline-injected rats (Mean±SEM, n=7).

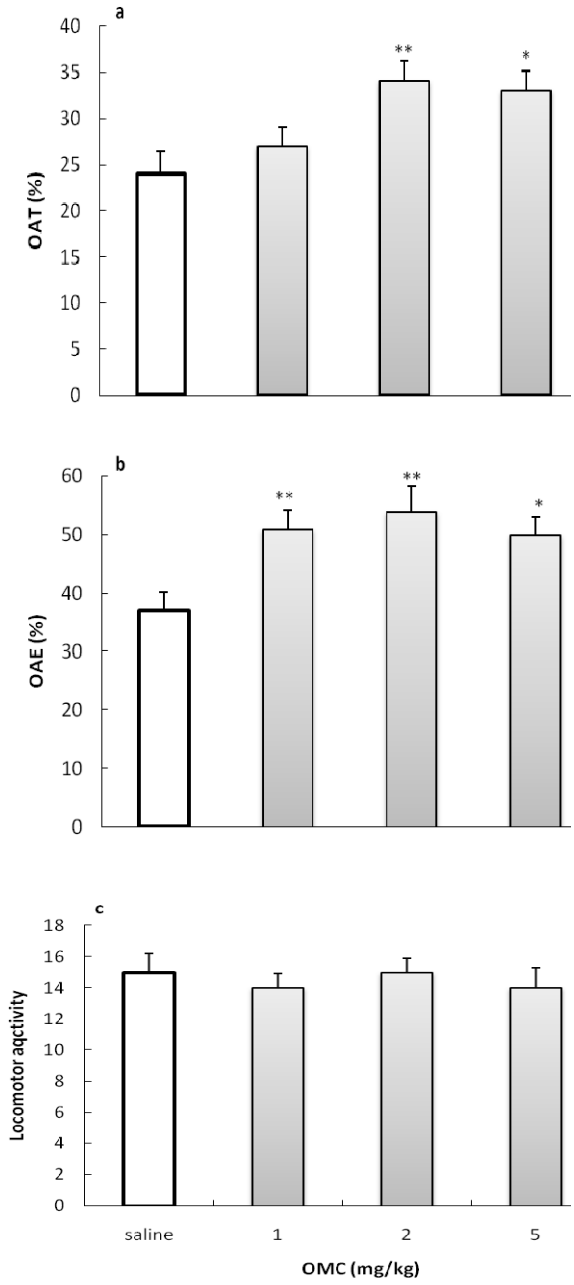


Figure 3. The effects of IP injection of OMC (1, 2 and 5 mg/kg) on %OAT (a), OAE (b) and locomotor activity(c). **P*< 0.05 and ***P*< 0.01 compared with saline-injected rats (Mean±SEM, n=7).

Discussion

Results of present study demonstrated that methyl and methoxy hydroxyl derivatives of phencyclidine (PCP) (I), [(1-[1-(4-methylphenyl) (cyclohexyl)] 4-piperidinol, (MC) (II) and 1-[1-(4-methoxyphenyl) (cyclohexyl)] 4-piperidinol, (OMC) (III) reduced the anxiety-like behaviors exhibited by specific increases in the percentage of open arm time (%OAT) and percentage of open arm entries (%OAE). Our data also showed that IP administration of MC and OMC increased both %OAT (%Open Arm Times) and %OAE (%Open Arm Entries)-the parameters of anxiety-related behavior-without locomotor impairment in the elevated plus maze. Such finding indicates that the induction of anxiolytic response by MC and OMC PCP acts primarily as an NMDA receptor antagonist, which blocks the activity

of the NMDA receptor (7-9). Recent developments in the neurobiology of anxiety have highlighted the neurotransmitter glutamate as an important element in anxiety and anxious behavior. The effect of glutamate on anxiety behaviors are mediated through different combinations of ionotropic as well as metabotropic glutamate receptors and potentially different sub-unit combinations. (22-24).

Anxiolytic like effects of antagonists of metabotropic glutamate receptors have been reported in several studies. The hippocampus may be involved in these anxiolytic-like actions, because the intrahippocampal injections of LY354740, mGLU2/3 receptor agonist produced anxiolytic-like effects in rat models. Furthermore, in mice the anxiolytic-like activity of LY354740 was associated with the suppression of EPM-induced c-Fos in the hippocampus (23-25).

The major research efforts so far have been directed towards the development of compounds which modulate the function of NMDA receptors by acting within the NMDA receptor complex. The utility of NMDA antagonists appeared to be greatly hampered by adverse effects on anxiety, because of interference with receptors throughout the whole CNS and body (26). Some studies have shown the anxiolytic-like effects of NMDA-receptor antagonists. Systemic or intra-dorsolateral PAG administration of NMDA-receptor antagonists produces anxiolytic-like effects in several animal models of anxiety, including the elevated plus-maze (EPM), brain aversive electrical stimulation and Vogel's punished licking test (27, 28).

PCP analogues have been shown to inhibit nicotinic acetylcholine receptor channels (nAChR) in rats (29-30). The recent studies also showed that NMDA receptor antagonists PCP have direct effects on serotonin (5-HT) receptors and systemic PCP treatment elevates brain extracellular 5-HT level by interaction with 5-HT reuptake site (31, 32). Serotonergic and nicotinic cholinergic systems have been extensively implicated in an array of behavioral and physiological functions including the control anxiety behaviors (21, 33). Therefore it seems that all of the NMDA glutamatergic system, nicotinic acetylcholine receptors and serotonin (5-HT) receptors could have a role on modulation of anxiolytic effects of phencyclidine and its derivatives (21, 28, 30-33).

According to above mentioned argument, different brain systems and receptors are involved in modulated behavioral effects of PCP and its analogues. Since there was not any report about effect of PCP on anxiety behaviors, we applied two derivatives of this molecule with the changes in substitution on its phenyl and piperidine rings (II, III, scheme 1) with more hydrophilic, polarity, electron distribution and dipole moments properties (35, 36) to increase the anxiolytic effects.

It seems that strong electron donating properties of the methyl group on *para* position of phenyl ring as well as hydrophilic and polarity properties of hydroxyl group on the piperidine ring of the molecule (II) facilitate and alter interactions with receptors. It is also anticipated that food and water intake could be increased as compared with PCP and vehicle (control). Also strong electron donating properties of the methoxy group on *para* position of phenyl ring and hydrophilic and polarity properties of hydroxyl group on the piperidine ring of the molecule (III) increased anxiolytic function in comparison to the PCP and vehicle (control). However because of undesirable reactions with cationoid intermediates (37), minor decrease in receptor binding could be anticipated. This increase is smaller than II but still higher than PCP and vehicle (control).

Conclusion

This study revealed that either of the two derivatives of phencyclidine (II, III) was more effective than PCP in modulation of anxiety behavior in rats and appropriate substitution of the methyl, methoxy and hydroxyl groups may result in ligands with different interaction for the PCP site on receptors.

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