



Interaction of Opioid Receptors in Anticonvulsant Effect of Progesterone in Ovariectomized Mice

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ABSTRACT

Background: It is well known that progesterone (i.p.) have a potent anticonvulsant effect in human and animal. This study was designed to evaluate the involvement of opioid receptors in this effect of progesterone in ovariectomized mice. **Methods:** All animal received intraperitoneal injection of drugs (progesterone and naloxone) two week s after surgery, for ovariectomy. Convulsion induced by subcutaneous injection of drugs. Onset, Number, death time and duration of convulsion were recorded for evaluation of the convulsion. **Results:** Progesterone (25, 50 mg/kg i.p.) decreased the convulsive symptoms. Anticonvulsant effect of high dose of progesterone was abolished by naloxone (5 mg/kg i.p.). Administration of the same doses of the naloxone, did not alone affect strychnine- induced convulsion. **Conclusion:** These results suggest that central opioids receptors may be play an important role in the anticonvulsant effect of progesterone.

Introduction

Steroid hormones produced by the ovaries, placenta, testes, adrenal glands.¹ These hormones as neurosteroid produce in brain and spinal cord too.² They affect CNS (central nervous system) fncion through glia and neurons include neurotransmitter synthesis, regulation receptor expression and synaptic transmission.³

Recently progesterone has been greatly studied within the confines of reproductive function and also as a neurosteroid.^{1,4,5} Such hormones are involved in numerous aspects of brain function with autocrine or paracrine signaling mechanisms⁶ via their intracellular nuclear and membrane receptors.^{2,7} It has been reported that progesterone has sedative, antinociceptive, anesthetic, and anticonvulsant properties in animals. This hormone decreases the frequency of seizures in women with epilepsy.^{1,8,9} Structures with significant role in seizure such as hippocampus are important targets of this hormone.¹⁰⁻¹²

Convulsive model could be induced in animals by electroconvulsive shock¹³ or injection of chemical drugs such as: bicuculline,¹⁴ picrotoxin,¹⁵ kainic acid,^{16,17} pilocarpine,¹⁸ isoniazid,¹⁹ pentylenetetrazole (PTZ)^{9,13} and strychnine.²⁰⁻²² These models apply for study effect of drugs on convulsion.

There is considerable evidence supporting a protective role for opioids against several distinct models of

epileptic disorders such as seizures induced by electroconvulsive shock^{19,23,24} and temporal lobe epilepsy.²⁵ Morphine -as an opioid agonist- in a low dose show an anticonvulsant effect against seizures induced by picrotoxine and pentylenetetrasol.^{23,26,27} Opioid mechanism modulates seizures induced by GABA transmission blockers.^{24,28,29}

Several studies have been conducted to determine the mechanisms underlying the anticonvulsant effect of progesterone.²⁸ Rapid effect of progesterone is associated with membrane receptors.^{7,28} Anticonvulsant effect of progesterone is believed that mediated by different neurotransmitter system such as GABA-ergic^{2,28,29} and glutamatergic²⁸ system and through nongenomic or genomic modulation.^{8,28} Some investigation indicate the interaction of progesterone with neurotransmitter system such as cholinergic and opioidergic in CNS.^{2,28,30} A different study reported interaction between progesterone action and opioidergic system.^{11,30-37}

According to these interactions between progesterone and opioid system that reported above, the aim of this study is to investigate the interaction between anticonvulsant effect of progesterone and opioid receptor in strychnine induced convulsion model in ovariectomized mice.

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Materials and Methods

Animals

In this study female mice (laboratory animal's center, Ahvaz, Iran) weighing 20-35 g was used. Animals were housed under standard laboratory conditions that included controlled ambient temperature ($21 \pm 2^\circ\text{C}$), a 12-hour-dark/12-hour-light (7:00 a.m. to 7:00 p.m.) cycle. The animals were randomly distributed into different groups.

The animal care was provided under the supervision of a qualified veterinarian. This study was performed under control of Iranian Society for the Prevention of Cruelty to Animals.

Ovariectomy

For the lack of the cyclic change effect of steroid hormones, the female mice were ovariectomized. Animals were initially injected i.p. with ketamine (70mg/kg) and xylazine (7 mg/kg) until loss of consciousness and loss of any response. Ovaries were then cut away from the uterus and the uterus was allowed to settle back into the abdominal cavity. Subsequently, the skin closed. All subjects were allowed 14 days for recovery before experiments commenced.

Drugs

Progesterone was purchased from Iran Hormone Co. (Tehran, Iran). Strychnine was purchased from Sigma Chemical Co (USA). The vehicle Almond oil was obtained from Barige Esanse Co. (kashan, Iran). Naloxone hydrochloride was purchased from Iran Tolid Daru Co. (Tehran, Iran). Ketamin hydrochloride and Xylazine hydrochloride vials purchased from Alfasan Pharmaceuticals Holand. Progesterone was dissolved in Almond oil and other drugs were dissolved in saline. All drugs were administered intraperitoneally (i.p.) except Strychnine which was given subcutaneously (s.c.). Drugs injected at a volume of 5 ml/kg of mouse body weight. All experiments were performed between 12:00 and 19:00 h.

Protocol of study/ Experiments

In this study convulsion induced by strychnine as other studies.^{27,28} All animal received s.c. strychnine (1mg/kg) 30 min after drugs. Seizure parameters include onset and death time of convulsion were assessed for 30 min after strychnine injection. The effect of drug injection on seizure parameter was evaluated.

Our protocol for testing the effect of opioid receptor on anticonvulsant effect of progesterone: the ovariectomized mice were divided into three series of experiments.

All the procedures were carried out in accordance with institutional guidelines for animal care and use.

First series of experiment

In this experiment anticonvulsant effect of progesterone is evaluated.

Three groups of animal received different dose of progesterone (0, 25, 50 mg/kg) 30 min before injection of strychnine.

Second series of experiment

In this experiment the effect of naloxone on convulsion is evaluated.

Four groups of animal received different dose of naloxone (0, 1, 2, 5 mg/kg) and strychnine injected subcutaneously 30 min later.

Third series of experiment

In this experiment the effect of interaction of naloxone with anticonvulsant effect of progesterone is evaluated.

Four groups of animals were pretreated with acute administration of different dose naloxone (1, 2, 5 mg/kg) then at 15-min intervals they received high doses of progesterone and strychnine injected subcutaneously 30 min later.

Data Analysis

Data are presented as mean \pm S.E.M. Significance of differences for the seizure parameters between groups assessed using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc comparison. The significance level was defined as $P < 0.05$.

Theory

Several studies have been conducted to determine the mechanisms underlying the anticonvulsant effect of progesterone. The effects of progesterone are believed that mediated by different mechanism. Regarding that various studies have reported the interaction between progesterone and opioid system which interfere in seizure, but there still no significant study about the interaction between opioid receptor and anticonvulsant effect of progesterone. Thus aim this study is to investigate interaction between progesterone and opioid receptor.

Results

Figure 1 illustrates the effect of different doses of progesterone (0, 25, 50 mg/kg) on onset duration and death time and number of convulsion in strychnine-induced convulsion in OVX mice. One-way ANOVA revealed a significant effect. Post hoc analysis showed that progesterone significantly increased onset ($P < 0.001$) and death time ($P < 0.01$) of convulsion and decreased number of convulsion ($P < 0.05$) dose dependently.

Figure 2 illustrates the effect of naloxone (0, 1, 2, 5mg/kg) on onset duration and death time and number of convulsion in strychnine-induced convulsion in OVX mice. One-way ANOVA analysis showed no difference in seizure parameters between groups receiving naloxone.

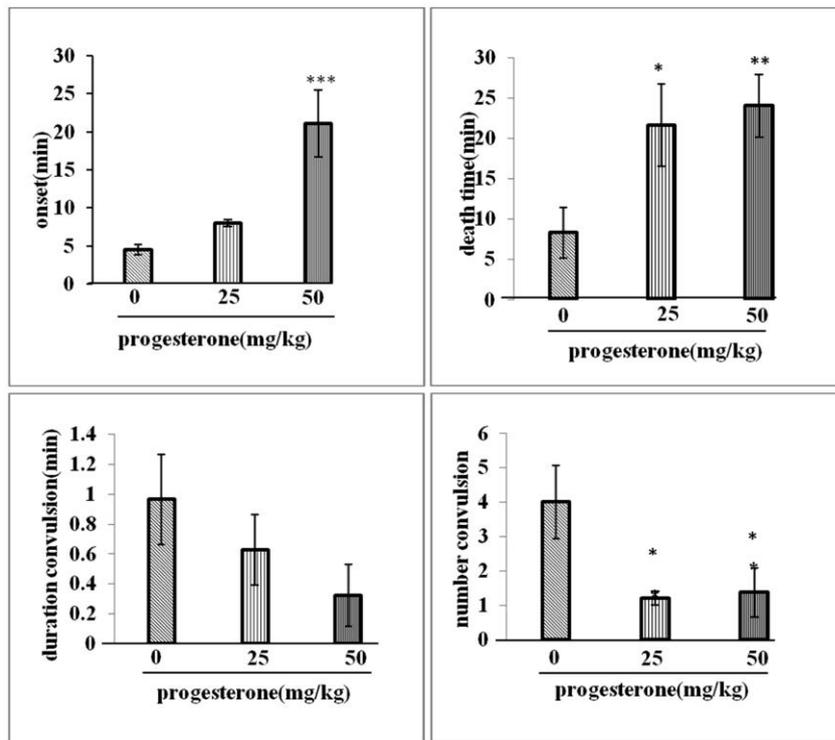


Figure 1. Effect of progesterone on strychnine-induced convulsion in OVX mice: All animal received s.c. strychnine (1mg/kg) 30 min after progesterone (0, 25, 50 mg/kg) -control shown as 0-, then seizure parameters evaluated for 30 min period immediately. Columns represent means \pm SEM in each group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with vehicle-treated group (control).

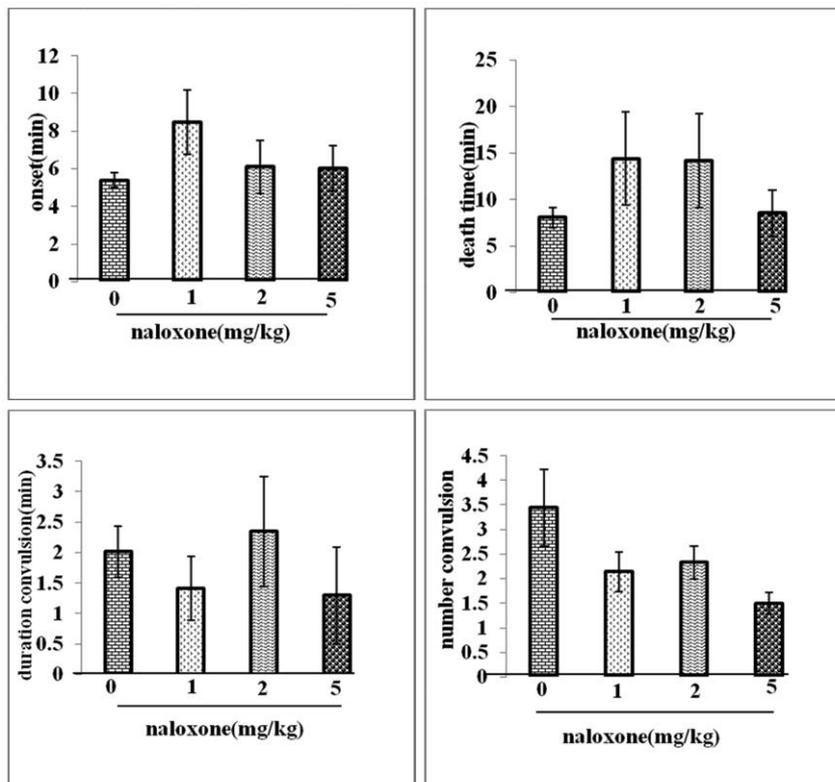


Figure 2. Effect of naloxone on strychnine-induced convulsion in OVX mice: All animal received s.c. strychnine (1mg/kg) 30 min after different doses of naloxone (0, 1, 2, 5mg/kg), then seizure parameters evaluated for 30 min period. Columns represent means \pm SEM in each group.

Figure 3 illustrates the effect of interaction of different doses of naloxone (1, 2, 5mg/kg) with high dose progesterone (50 mg/kg) on onset duration and death time and number of convulsion in strychnine-induced convulsion in OVX mice. One-way ANOVA analysis showed that naloxone (5mg/kg) decreased onset ($P < 0.$

01) and death time ($P < 0. 01$) of convulsion in progesterone-treated group. Pretreatment of several doses of the opiate antagonist naloxone with progesterone is obtained a dose-dependent inhibition of anticonvulsant effect of progesterone.

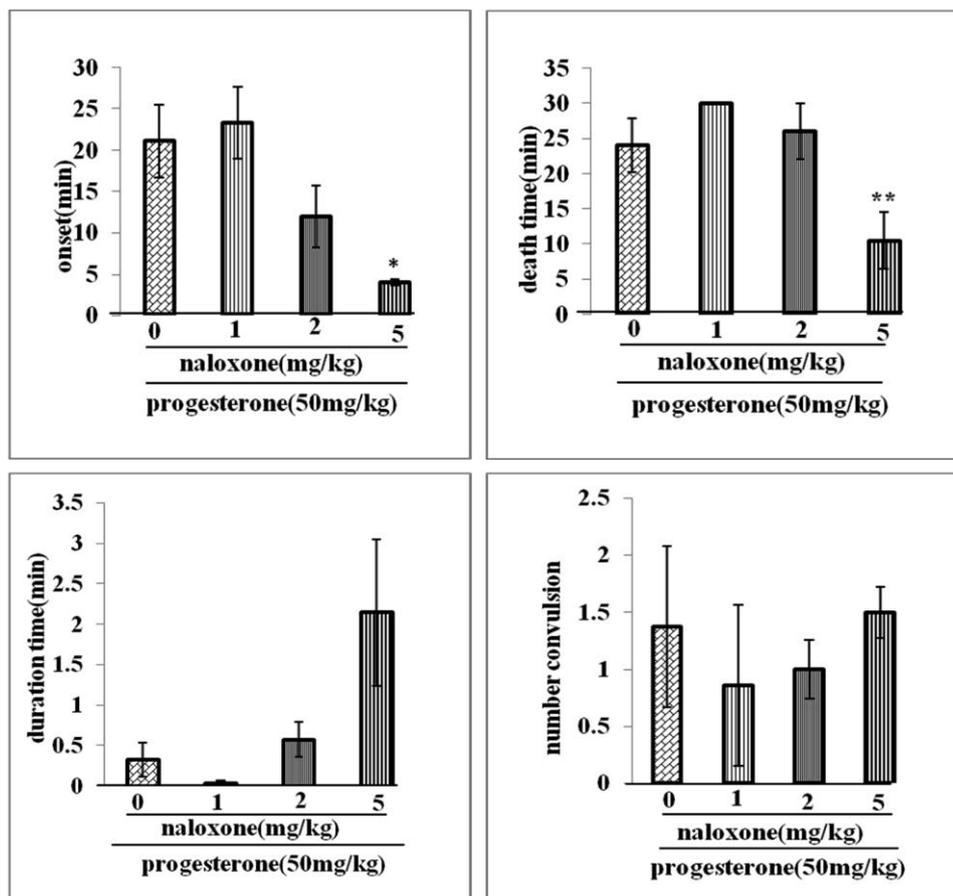


Figure 3. Interaction of naloxone with anticonvulsant effect of progesterone on strychnine-induced convulsion in OVX mice: animals received naloxone (0, 1, 2, 5mg/ kg) and progesterone (50 mg/kg) before injection of strychnine 45 min and 30 min respectively. ** $P < 0. 01$ compared with progesterone-treated group (shown as 0).

Discussion

In this study the interaction between anticonvulsant effect of progesterone and naloxone is evaluated. We showed for the first time that the anticonvulsant activity of progesterone can be decreased by naloxone dose dependently at a dose that has no effect on convulsion.

In our experiments corresponding to the other studies the strychnine injection in ovariectomized mice lead to convulsion.^{2,28,37-39}

Rapid anticonvulsant effect of progesterone is probable associated with membrane receptors nonclassical rapid signaling events induced by membrane receptors.

It seems that progesterone functions in brain directly or indirectly mediated by different neurotransmitter mechanisms such as GABAergic,^{2,28,29} glutamatergic,^{5,28,33} opioidergic.³⁰ Progesterone modulates GABA and glutamate receptor by its

metabolites such as allopregnanolone and exerts its antinociceptive and antianxiety effects.^{28,40}

The anticonvulsant effect of progesterone may be caused by presynaptic inhibition of glutamate release in the hippocampus³³ or interaction with GABA receptors.²⁹ Studies have shown that opioid receptors play an important role in modulating seizure parameters. This effect of opioids is mediated by inhibiting the stimulating pathways and increasing GABAergic activity.²⁷

Morphine shows different effects on the seizure threshold based on doses and model of seizure.⁵ Opioid antagonist naloxone has no significant effect on the strychnine-induced convulsion symptoms solely and in the doses used in this study. There are different reports about the effect of naloxone on seizure in various models. Some studies have shown the ineffectiveness of naloxone in low doses.⁴¹ On the other hand, they showed that intracerebral injection of naloxone causes seizure^{42,43} while this drug in low

doses reduces seizure symptoms in some models.⁴⁴ Different effect of naloxone might depend on the drug dose and seizure model in experimental animals. The amounts of naloxone in this study which has no effect on strychnine-induced convulsion reduces the anticonvulsant effect of progesterone. So it seems that central opioid receptors involved in anticonvulsive effect of progesterone.

The progesterone effect and opioid system interaction has been reported in some physiological processes such as pain.^{29,36} Also, both systems are involved in regulating nervous system stimulation.²⁸ On the other hand, regarding that at least a part of progesterone effects and opioid receptors is mediated by GABAergic system. It is probable that opioid receptors interference in progesterone anticonvulsive effects through interaction to GABAergic receptors function such as GABA receptor A.^{27,29}

On the other hand, nitric oxide (NO) plays an important role both in opioid system modulating effects on anxiety⁴⁵ and also it has been characterized as one of the probable mechanisms of progesterone anticonvulsive effect.²⁸ Thus opioid receptors via nitric oxide system might interfere in progesterone anticonvulsive effects.

Considering the different central neurotransmitter system modulated in progesterone physiological effects in nervous system,²⁸ it is probable that naloxone reduces progesterone anticonvulsive effect by reducing opioid system performance and interacts to these neurotransmitter systems activities.

Conclusions

In this study the interaction between anticonvulsant effect of progesterone and opioid receptor is evaluated. Regarding to results of this study and other investigation, it seems that opioid receptor play a role in anticonvulsant effect of progesterone. This effect is probably exerted directly via the interaction between progesterone function with opioidergic neurons or modulate indirectly via interaction to other systems such as GABAergic, nitric oxide, cholinergic and glutamatergic.

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