Research Article

Inhibitory Effect of NMDA Receptors in the Ventral Tegmental Area on Hormonal and Eating Behavior Responses to Stress in Rats

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Background. Stress and its consequences are among the causes of accidents. Objective. The effects of intraventral tegmental area (I-VTA) memantine on the plasma corticosterone and eating parameters disturbance induced by acute stress were investigated. Methods. Male Wistar rats (W: 250–300 g) were divided into control and experiential groups, each of which received memantine either intra-VTA or peripherally. One week after bilateral cannulation, the rats received memantine (1 and 5 μg/Rat) five min before electroshock stress. The other experimental groups received memantine (1 and 5 mg/kg) intraperitoneally 30 min before stress. The control groups received saline or memantine but did not experience stress. Food and water intake and plasma corticosterone level were recorded. Results. Results showed that stress decreases food intake but does not change water intake and increase in plasma corticosterone level. Intraperitoneal memantine administration slightly inhibits the stress effects on food intake. However, water intake and plasma corticosterone level were increased. Intra-VTA memantine reduces the effects of stress on corticosterone and water intake. Conclusion. It could be concluded that inhibition of glutamate NMDA receptors in the VTA by memantine leads to the inhibition of the eating behavior parameters and plasma corticosterone level disturbance induced by stress in rats.

1. Introduction

It is now clear that exposure to stressful events elicit sever consequences in the veterans; despite intense investigations, the exact mechanisms underlying these effects are not fully understood [1]. The ventral tegmental area (VTA) of the midbrain contains the cell bodies of the mesolimbic dopamine system which serves as a target for molecules released during stress [2–4]. A considerable body of research also indicates that glutamatergic neurotransmission within the ventral tegmental area might be a critical neurochemical determinants of stress effects [5–9]. Glutamate induces its effects in part by activation of its N-methyl-D-aspartate (NMDA) receptors, which are extensive distribution in the VTA [9, 10]. Activation of NMDA receptors stimulates the entry of calcium ions into the cells and thus increases the activity of dopamine neurons and also the stimulation of dopamine secretion in the target areas of these neurons, which were described above [11–13]. Glutamatergic projections from the medial prefrontal cortex are the main glutamate sources
to the VTA and it seems that these inputs along with the
dopaminergic projections from ventral tegmental to nucleus
cumbens, amygdala, and prefrontal cortex modulate the
activity of stress system in the animals [6, 7, 9, 10, 14–21].

Various investigations indicate that glutamate system
may have a role in modulating stress side effects. In this
regard, studies indicated that inhibition of NMDA receptors
decreases stress side effects [22, 23]. These studies indicated
that peripheral injection of memantine in the rats subjected
to chronic mild stress for seven days inhibit stress-induced
anorexia, adrenal gland weight excess, and plasma corti-
costerone elevation. The drug also decreases BDNF levels
elevation induced by stress in the prefrontal area of cerebral
cortex [22, 23]. On the other hand, it has been found that
memantine injection prevents stress-induced cell production
decline in the hippocampus. In this regard, restraint stress
applied for 2 h/day for seven days reduces cell proliferation
in hippocampus which was inhibited by memantine [24].

Moreover, memantine may also be useful in treatment of
posttraumatic stress disorder (PTSD) both in animal models
and human [25–27]. Considering the conducted researches,
in the present study, the fact that the effect of memantine
injection either peripherally or into VTA on plasma corti-
costerone levels and some eating behavior parameters such as
food and water intake and delay to eating changes after acute
stress in male Wistar rats was investigated.

2. Materials and Methods

2.1. Animals. Wistar rats (W: 250–300 g) obtained from Iran
Pasteur Institute were used in this study (4 rat/cage). The
animals were not fasted before the test and used standard
food and water. The animals were kept in their home cages
for one week before the experiments and their food and
water intake were measured as the base of the food and water
intake. Dark/light period of 12/12 h and humidity of 50%
were conducted for the animals. All the experiments were
conducted according to Animal Rights Guidelines, Deputy
of Research, Baqiyatallah University of Medical Sciences. In
each series of experiments, 8 animals were used.

2.2. Drugs. Memantine hydrobromide (Sigma, USA), keta-
mine hydrochloride, and diazepam (Sigma, USA) were used
in this study. All drugs were dissolved in sterile saline and
injected i.p. at the dose of 1 mL/kg. For I-VTA injections,
memantine was dissolved in the sterile saline and injected
to the animals in volume of 0.25 μL/Rat. Since ketamine
hydrochloride is a NMDA receptor antagonist, after the
surgical processes, each animal was allowed one week for
recovery from the ketamine and surgery.

2.3. Stress Induction. Stress box was used to perform this
experiment. This device has 9 compartments composed of
Plexiglas. The floor of the compartments had steel wires
and was connected to the electroshock device, which was
controlled by a PC. The severity and duration of electroshock
induction were 60 mV, 10 Hz, and for 100 Sec [28]. Animals
were placed in the apparatus 30 min before the stress begin-
ning and maintained in the apparatus after stress termination
for additional 30 min. In experimental groups, the animals
received 1 and 5 mg/kg intraperitoneally and/or 1 and 3 μg/rat
intra-VTA memantine 5 min before stress induction. Blood
samples were taken after stress termination in order to
measure the plasma corticosterone concentration as stress
index in these animals.

Animals of the control groups were treated similar to the
ones in the case group, but they received saline instead of
memantine. After the termination of stress, the animals were
returned to their cage and time interval between their return
and the beginning of feeding was measured and declared as
delay to eating index. Moreover, food intake of each animal
was measured as another behavioral indicator. Blood samples
were taken from retroorbital sinus of the rats before or after
stress termination.

2.4. Surgical Procedures. To inject memantine into VTA, the
animals were first anesthetized by intraperitoneal injection
of ketamine (50–75 mg/kg) and diazepam (5–7 mg/kg). Two
stainless steel guide cannulas (Gauge 21) were bilaterally
placed within the VTA. These cannulas were fixed using two
screws and dental acrylic. Coordinates for cannulas were as
follows: AP: −4.8 mm from Bregma, L: 1 mm from central
line, and V: 7.5 mm from the surface of skull [29]. Injection
into this area was performed by a stainless steel injection
cannula number 30 which was attached to a 5 μL Hamilton
syringe by a plastic cannula (injection volume was 0.25 mL
on each side). After the tests termination the animals were
deply anesthetized b ketamine (100 mg/kg) and their brains
were fixed using Trans cardiac fixation method. The site of
injection was specified by a specialist.

2.5. Histology. After the completion of testing, all animals
were anesthetized and received a transcardiac perfusion
with 0.9% normal saline followed by 10% buffered formalin.
The brains were removed, blocked, and cut coronally in
40 μm sections through the cannula placements. The tissues
were stained with cresyl violet and were examined by light
microscopy by an unfamiliar observer to the behavioral data.
Only the animals with correct cannula placements were
included in the data analysis (Figure 1).

2.6. Statistical Analysis. Data were expressed as Mean ±
SEM. In order to analyze the data, two-way analysis of
variance (two-way ANOVA) was applied for the analysis
of the differences between memantine treated groups using
memantine and stress as factors. When two-way analyses
showed a significant difference, the Tukey post hoc were used.
P < 0.05 was considered significant.

3. Results

3.1. Effects of Peripheral and/or I-VTA Memantine on Plasma
Corticosterone Level after Stress. In this series of experiments,
12 groups of animals (n = 7/group) were used. Six groups
of them underwent surgery and cannulation as mentioned
in the Materials and Methods section; after 7 days of recovery, they were exposed to electrical foot shock. Test groups received bilateral different doses of memantine (1 and 5 μg/rat) or saline (in the same volume) within their VTA (0.25 μL/rat/side) 5 min before stress induction. The control groups received the same dosage of memantine or saline without stress exposure. Other six groups received memantine (1 and 5 mg/kg) or saline (1 mL/kg) intraperitoneally with or without stress exposure. The results indicated that stress dramatically increases plasma corticosterone level and memantine administration reduces the stress effects both when injected as IP or I-VTA (two-way ANOVA within group comparison: memantine effect: F(9, 63) = 3.21, P < 0.01; stress effect: F(1, 63) = 2.79, P < 0.05; memantine × Stress effects: F(9, 63) = 4.36, P < 0.01)(Figure 2).

3.2. Effect of Peripheral or I-VTA Bilateral Memantine Administration on Food and Water Intake after Stress. Our data indicated that memantine by itself increases or decreases food and water intake when injected peripherally or I-VTA, respectively (Figures 3 and 4). However, stress decreased food intake and did not change water intake in the animals (Figures 3 and 4). Treatment with memantine increased food intake under both nonstress and stress conditions (two-way ANOVA within group comparison: memantine effect: F(9, 63) = 4.18, P < 0.01; stress effect: F(1, 63) = 5.68, P < 0.01; Memantine × Stress effects: F(9, 63) = 5.09, P < 0.01) (Figures 3 and 4).

3.3. Effect of Peripheral or I-VTA Memantine Administration on Delay to Eating Induced by Stress. In this series of the experiments, the above-mentioned groups were returned to their cages 30 min after the termination of stress and their delay to eating was measured. The results indicated that in the negative control group (which were placed in the stress box but not exposed to stress), delay to eating time was very short after the animals were returned to their home cages (Figure 5). This time was dramatically increased for control stress group (Figure 5). However, the time to initiating the eating in the animals which received memantine (either IP or I-VTA) was increased significantly (two-way ANOVA within group comparison: memantine effect: F(9, 63) = 4.79, P < 0.01; stress effect: F(1, 63) = 3.19, P < 0.01; Memantine × Stress effects: F(9, 63) = 6.3, P < 0.01) (Figure 5).
4. Discussion

The present study was designed to respond to the question whether a modulation of NMDA glutamate receptors within the VTA ameliorates the plasma corticosterone levels and some eating behavior parameters changes induced by an uncontrollable stress or not. The findings however have shown that inhibition of these receptors inhibit the stress effects and promote the overall animal status.

Several studies have shown that stress induces several hormonal responses from which the corticosterone increment was considered as the main response [1]. Our data indicated that corticosterone plasma level was increased in the stressed animals. In agreement with our finding there are data indicating that different kinds of stress including the method which is used in the present study increase corticosterone plasma level in both human [30] and rats [31]. Plasma corticosterone increment is postulated to be the result of hypothalamus-pituitary-adrenal axis activity [1]. Surprisingly, the plasma corticosterone level in the animals which received memantine also increased significantly. In agreement with our finding, Rêda et al., 2012, have shown that memantine in rats increases plasma corticosterone slightly [23]. However, the researchers used the dose of 20 mg/kg of the drug, whereas the dose of 10 mg/kg was ED 50% in our study. One possible reason for the different data may be due to different type of animals used in these two studies [23]. It is important to be mentioned that in some cases the lower dose of memantine was more effective.

The exact mechanism(s?) by which memantine induces corticosterone release is not understood. It must be noted that memantine increases plasma corticosterone and adrenocorticotropine hormone (ACTH) levels in the rats [32]. Our data indicated that at least one of the brain sites for memantine effects on corticosterone may be the ventral tegmentum area. However, the precise neuroanatomical relationship between VTA and the adrenal gland is not understood and remains to be examined. Our data also indicated that memantine affects the brain reward system and also manipulation of brain reward system interacts with stress system, the fact that was mentioned in several studies [8]. Studies have indicated that glutamate NMDA receptors are located on the cell membrane in the adrenal cortex and inhibition of these receptors may increases the corticosteroids release from these cells [33]. Similar mechanism may be involved in the results obtained in the present study for memantine. However, it is not understood why administration of memantine inhibits stress induced corticosterone release. One possible mechanism is that memantine may inhibit central glutamatergic mechanisms involved in the mediation of stress-induced CRF release. This hypothesis is supported by the fact that all of the stress side effects which are related to the CRF including food intake inhibition and anorexia also were inhibited by memantine pretreatment in the present study.

Memantine treatment either intraperitoneally or intra-VTA in both stressed and nonstressed groups in different doses increased delay to eating time in the animals. It is an interesting finding and there is no data for discussion in this regard. However, it is clear that memantine even potentiates the stress effect in this regard. It is not clear from our finding how the drug can induce such responses but it may function between different parts of the brain which are involved in response to stress with memantine.

As a general conclusion, it seems that intra-VTA and peripheral administration of antagonists of NMDA glutamate receptors (memantine) could decrease plasma corticosterone levels elevation as well as eating behavior parameters such as food and delay to eating changes caused by foot electrical
shock stress in animals; this effect could be due to the central and also peripheral effects of memantine.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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**References**


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