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Original Article

Acute and chronic tramadol administration impair spatial memory in rat

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Abstract

Tramadol hydrochloride, a synthetic opioid, acts via a multiple mechanism of action. Tramadol can potentially change the behavioral phenomena. The present study evaluates the effect of tramadol after single or multiple dose/s on the spatial memory of rat using object recognition task (ORT). Tramadol, 20 mg/kg, was injected intraperitoneally (i.p) as a single dose or once a day for 21 successive days considered as acute or chronic treatment respectively. After treatment, animals underwent two trials in the ORT. In the first trial (T1), animals encountered with two identical objects for exploration in a five-minute period. After 1 h, in the T2 trial, the animals were exposed to a familiar and a nonfamiliar object. The exploration times and frequency of the exploration for any objects were recorded. The results showed that tramadol decreased the exploration times for the nonfamiliar object in the T2 trial when administered either as a single dose ($P<0.001$) or as the multiple dose ($P<0.05$) compared to the respective control groups. Both acute and chronic tramadol administration eliminated the different frequency of exploration between the familiar and nonfamiliar objects. Our findings revealed that tramadol impaired memory when administered acutely or chronically. Single dose administration of tramadol showed more destructive effect than multiple doses of tramadol on the memory. The observed data can be explained by the inhibitory effects of tramadol on the wide range of neurotransmitters and receptors including muscarinic, N-methyl D-aspartate, AMPA as well as some second messenger like cAMP and cGMP or its stimulatory effect on the opioid, gama amino butyric acid, dopamine or serotonin in the brain.

Keywords: Tramadol; Acute; Chronic; Memory; ORT

INTRODUCTION

Tramadol hydrochloride is a centrally acting synthetic opioid (morphine like drugs), acts through a dual mechanism of action (1,2). It stimulates the mu-opioid receptors or inhibits the serotonin and norepinephrine reuptake which explains its antidepressant effects (3-5). Clinically active tramadol is a racemic mixture of two enantiomers that have two distinct but complementary mechanisms of action: the (+) tramadol is a selective agonist of mu-opioid receptor which preferentially inhibits serotonin reuptake and enhances serotonin efflux in the brain, whereas the (-) enantiomer mainly inhibits noradrenaline reuptake (6). In fact the major metabolite of both enantiomers of tramadol (O-desmethyl–Tramadol) is more potent to stimulate mu-opioid receptor than the parent compound (5,7).

Tramadol is used to alleviate the moderate and severe pain (8). Because of its lower susceptibility of addiction than morphine, it is commonly used for patients in the postsurgical period and also in patient with chronic pain syndromes like rheumatoid arthritis (9), renal colic pain (10), back pain (11), neuropathic pain and fibromyalgia (12).

Tramadol is chronically used to treat the morphine and other opioids withdrawal syndrome (13). The mixed and specific

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mechanism of action has made tramadol as a potent drug of abuse especially in young adults (14,15). In some cases, tramadol is occasionally used to treat the premature ejaculation (16). So the use of tramadol, either for clinically aspects or its abuse has been dramatically increased during the last decades (3,14).

Memory formation is a complex process that requires distinct neuronal networks and multiple pre- and post-synaptic events. Several studies have been conducted to investigate the molecular mechanisms underlying activity-dependent synaptic changes during memory formation (17,18). These studies show that different neurotransmitter in the central nervous system (CNS) play a key role in the learning and memory. Stimulation of gama amino butyric acid (GABA) receptor or the increase of GABA release could impair the memory (19-21). As well, serotonergic neurons play a significant role in the learning and memory processes (22-24). It has been well known the amplification of noradrenergic and cholinergic system in the CNS improves the learning and memory in amnesia and Alzheimer's disease (17,25). The augmentation of dopaminergic system with its different agonist showed controversy in the memory formation (26-28). There are several studies illustrated that activation of opioid receptor impairs the memory in animals or in the human (29,30). In some researches, the antidepressant which increases the synaptic dopamine, norepinephrine and serotonin inverted the depression memory impairment (22,31).

The chronic or acute use of opioid agonist like morphine could impair the memory by the activation of mu receptor either in a direct or an indirect manner (29,32-34).

Although there are several reports in the literatures on the effects of neurotransmitters agonist and antagonist in learning and memory, the effect of tramadol as a drug with different mechanism of action by affecting mu receptor, 5HT, serotonin, norepinephrine and dopamine has not been well studied. So this study was designed to evaluate and compare the effect of chronic and acute administration of tramadol on the memory of rat in the object recognition task (ORT).

MATERIALS AND METHODS

Animals

The experiments were carried out on 28 male Wistar rats (weighing 220–230 g) obtained from Animal House of School of Pharmacy at Isfahan University of Medical Sciences. Animals had free access to food and water before and through the experiments and were kept at a constant room temperature (22 ± 1 °C), under a 12-h light/dark cycle. All experimental procedures were conducted during the light phase of the cycle. All animal experiments were approved by the Ethics Committee of Isfahan University of Medical Science and performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals.

Apparatus and objects

The apparatus consisted of a circular arena, 83 cm in diameter and 40 cm high wall, made of white polyvinyl chloride (35,36).

We used two different sets of objects consisted of (1) a massive aluminum cube was made manually (10.0 × 5.0 × 7.5 cm) and (2) a massive aluminum cube with a tapering top (13.0 × 8.0 × 8.0 cm). Each object was available in triplicate. The objects could not be displaced by rats (36,37).

Drug administration

Tramadol hydrochloride (Sigma-Aldrich, Germany, CAS: 36282-47-0) solution was prepared freshly in saline. The animal was intraperitoneally (i.p) injected with 20 mg/kg tramadol either as a single dose (acute treatment group) or once a day for 21 consecutive days (chronic treatment group). Each respective control groups was treated in the same manner with normal saline. Drug injection was made between 8-10 A.M every day.

Experimental procedure

One week prior to the behavioral test, animals were handled daily and were adapted to the procedure; that is, they were allowed to explore the apparatus (without any objects) twice daily in 5 min period with 1 h interval (36-38).

Object recognition consisted of three clearly defined phases: a training session or first trial (T_1), a training-test interval, and a test session or second trial (T_2) (39). Ninety minutes after the completion of drug administration in both acute and chronic treatment, the T_1 trial was performed.

During the first trial (T_1) in the object recognition task, each rat was placed into the arena and exposed to two identical objects (A_1 and A_2) for a period of 5 min. Two objects were placed in a symmetrical position about 10 cm away from the wall. The rats were then returned to their home cage for a 1 h inter-trial interval. The entire arena was cleaned with alcohol (70%), both objects removed and one replaced with an identical familiar copy and one with a novel object. Then rats were returned to explore the familiar (A) and novel object (B) in the test session (T_2) lasting 5 min, too.

Exploration was defined as follows: directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose. Sitting on the object was not considered exploratory behavior. The exploration time (s) for each object in each trial was recorded and the following factors were calculated:

e_1, is the total exploration time of both objects in the first trial ($eA_1 + eA_2$)

e_2, is the total exploration time of both objects in the second trial (eA + eB)

d_2, is the discrimination index (eB - eA)/ (eB + eA)

d_2 is an index which indicates the discrimination between the new and the familiar objects. Its value vary between +1 and -1, where a positive score indicates more time spent with the novel object, a negative score indicates more time spent with the familiar object, and a zero score indicates a null preference. F, is the frequency of the object exploration. Another measure of the object recognition task is R (recognition) index which is the time spent to explore the novel object relative to the both objects exploration time and calculated as R = eB/(eB + eA).

In every session, animals that explored less than 7 s in the second trial (e_2<7) were excluded from analysis (35-38). To avoid the presence of olfactory trails, the objects were always thoroughly cleaned with alcohol (70%). Moreover, each object was available in triplicate so neither of the two objects from the first trial had to be used as the familiar object in the second trial. In addition, all combinations and locations of objects were used in a balanced manner to reduce potential biases due to preferences for particular locations or objects (36-39).

Statistical analysis

Results are expressed as Mean ± S.E.M. The data value of different factors indicating the memory was analyzed using a t-test or one-way analysis of variance (ANOVA) according to the number of comparing groups. For multiple comparisons Tukey post hoc tests was used. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Effect of acute and chronic administration of tramadol on d_2 index

A 20 mg/kg single dose i.p injection of tramadol, 90 min before the T1 trial in the ORT, significantly decreased (P<0.001) the d2 index in the T2 trial compared to the control. Daily i.p administration of tramadol at the dose of 20 mg/kg for 21 consecutive days (chronic administration) reduced the d_2 index significantly (P<0.05) in comparison to the control group.

Although tramadol could diminish the d_2 index both in acute or in chronic administration compared to their respective control groups, the single dose injection of tramadol reduced the d_2 index more pronouncedly than when it chronically administered in repeated daily dose (20 mg/kg) for 21 days (P<0.001) (Fig. 1).

Effect of acute and chronic administration of tramadol on R index

Intraperitoneal administration of 20 mg/kg single dose of tramadol 90 min before the T_1 trial significantly decreased (P<0.01) the R index in the T_2 trial compared to the control. Daily i.p injection of tramadol at 20 mg/kg for 21 consecutive days (chronic administration) reduced the R index significantly (P<0.05) in comparison to the control group.

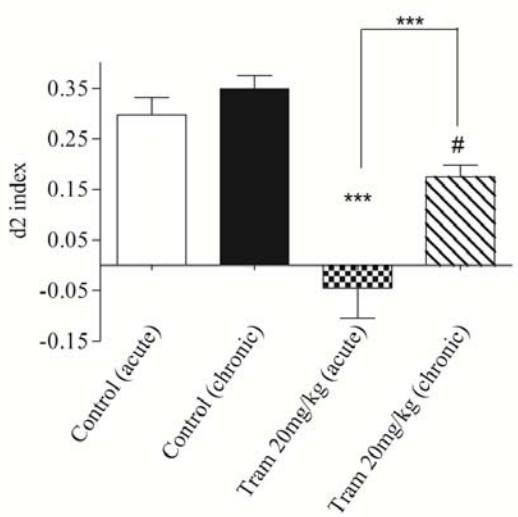

Fig. 1. The effect of chronic and acute administration of tramadol on the d2 index of exploration behavior test. A single i.p injection of 20 mg/kg tramadol 90 min before T1 trial significantly decreased (***P<0.001) the d2 index. Daily i.p injection of 20 mg/kg tramadol for 21 consecutive days (chronic administration) reduced the d2 index significantly (#P<0.05). Injection of a single dose of 20 mg/kg tramadol reduced the d2 index in the T2 trial more significantly than when it was administered daily for 21 days (***P<0.001).

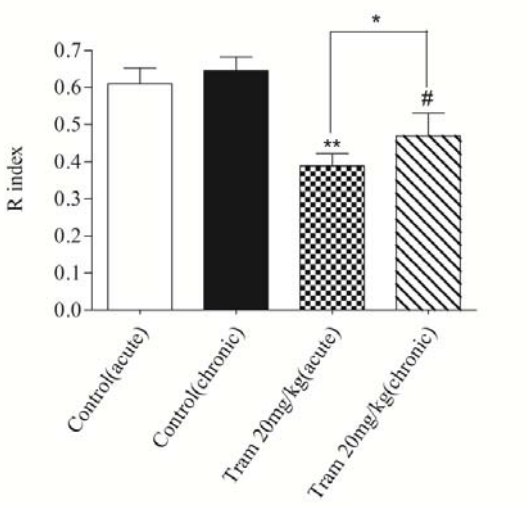

Fig. 2. The effect of chronic and acute administration of tramadol on the R index of exploration behavior test. A single i.p injection of 20 mg/kg tramadol 90 min before T1 trial in the object recognition task significantly decreased the R index(**P<0.01). Daily i.p injection of 20 mg/kg tramadol for 21 consecutive days (chronic administration) reduced the R index significantly (#P<0.05). Injection of a single dose of 20 mg/kg tramadol reduced the R index in the T2 trial more noticeably than when it was administered daily for 21 days (*P<0.05).

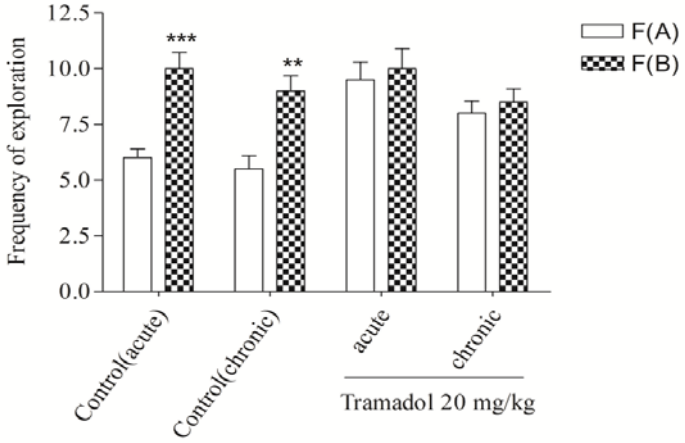

Fig. 3. The effect of acute and chronic administration of tramadol on the frequency of exploration. The frequency of novel object exploration compared to the old object was increased both in acute or chronic control groups significantly in the T2 trial (***P<0.001 and **P<0.01 respectively). But the animals who received 20 mg/kg tramadol acutely or chronically did not show any statistical differences.

Although tramadol could diminish the R index both in acute or in chronic administration compared to their respective controls, the single dose injection of tramadol reduced the R index in the T_2 trial more noticeably than when it chronically administered in repeated daily dose (20 mg/kg) for 21 days (P<0.05) (Fig. 2).

The effect of acute and chronic tramadol administration on the frequency of exploration

The frequency of novel object exploration increased both in acute or chronic control groups significantly compared to exploration frequency for the old object in the T2 trial (P<0.001 and P<0.01 respectively).

The animals who received 20 mg/kg tramadol acutely or chronically, however, did not show any statistically differences in exploration frequency for the new or old object in the T_2 trial (Fig. 3).

DISCUSSION

The current study was carried out on tramadol as an opioid analgesic drug that is increasingly used or abused for several purposes in a wide range of people. Tramadol belongs to the opioid analgesic drugs acting with different mechanism of action (3,4,14,15) which make it suitable for other indications like depression. The unique properties of tramadol allow administration of this drug in single or multiple doses for different disorders (6-10). Tramadol easily pass the blood brain barrier and reach a good level in the brain; thus it can potentially show behavioral changes (40). Previous human and animal studies have shown that morphine and other opioidergic agents can modulate learning and memory processes, either in positive or negative directions (25-27). The reported controversies may be due to different experimental conditions, protocols, and species used, and also variation in the dosage, route, and in particular the duration of the drug administration (41). Although there are a few studies on the effect of acute tramadol on the memory (42-44), researches are scarce on the effect of chronic tramadol. The ORT is based on the spontaneous behavior of rats to explore a novel object more intensely than a familiar one. It is a highly validated, non-rewarded, ethologically relevant test for recognition memory (45). In this study, we evaluated and compared the effect of acute or chronic administration of tramadol on the rat memory using ORT.

Our findings showed that 20 mg/kg single dose of tramadol decreased d_2 and R indices in the T_2 trial of ORT compared to the control group. These results indicated that the tramadol-treated group spent less time for exploration of the novel object than the familiar object. This treatment also did not show any difference for exploration frequency between the novel and old objects which was seen clearly in the control groups. Thus, according to the ORT protocol just a single dose of tramadol caused impairment on memory of the rats.

It was also found that daily administration of 20 mg/kg of tramadol for 21 consecutive days could decrease the d2, and R indices and frequency of exploration. Its chronic effect is relatively more moderate than the single dose on destructive effect of memory.

It is proved that different second messengers and neurotransmitter systems play key roles in the learning and formation of memory (17). The opioid receptor belongs to a well-known family of receptors called G protein–coupled receptors (GPCR) which in this case are linked to the Gi proteins (46-48). The stimulation of opioid receptor leads to the decrease of intracellular cAMP and cGMP. It is strongly documented that cAMP, cGMP and their related protein kinases have a basic role in the short and long memory (17,36,49). Tramadol as an agonist of opioid receptors can decrease the intracellular level of cAMP, cGMP, PKA, PKC and consequently neuroplasticity in the brain leading to the impairment of memory (50,51). A large volume of studies demonstrated that the opioid agonists will impair different kinds of memory (27,30,33,41,52-55). It has been shown that repeated administration of opioids may induce tolerance to its own response because of the receptor down regulation (56-58). This effect can explain the lower destructive effect of chronic administration of tramadol on the memory in our study.

One of the most important neurotransmitters in the improvement of memory is the cholinergic system act via the muscarinic system (17,50,51). Cholinergic antagonist like scopolamine diminishes the memory, whereas cholinesterase inhibitors and cholinomimetic agents are essential treatment of amnesia and Alzheimer's disease (59-62). Ragozzino and coworkers demonstrated that infusions of opioid agonists into the medial septum decreased hippocampal Acetyl Cholin turnover. Also it has been shown that intraseptal morphine administration, at a dose that impairs performance of memory, reduced Ach output in the hippocampal formation. The

results from a microdialysis analysis illustrated that administration of morphine into the medial septum decreased hippocampal Ach release (63). Shiraishi and colleagues, showed tramadol inhibited both the Ach-mediated response of M1 receptor *in vivo* and the decrease of the muscarine-induced accumulation of cGMP *in vitro*. However, the detail mechanism of M1 receptor inhibition by tramadol has not been well clarified (51). Our findings are in agreement with above mentioned reports showing decreasing effect of opioids on memory.

It has been shown that the stimulation of GABA receptor following administration of antiseizure drugs like phenobarbital diminishes the memory (20,64). The activation of opioid receptors will consequently increase the GABA activity. Thus the increase in the activity of GABA could be considered as a possible mechanism for the destructive effect of tramadol on the memory (19,65). Also it appears that the central cholinergic neurons are under inhibitory GABAergic control which means that the activation of GABA receptor will decrease the cholinergic activity in the CNS. These GABA receptors may be located pre- or post synaptically on Ach neurons (63,66,67).

It is well-known that the NMDA and AMPA receptors play a key role in the formation of memory and neuroplasticity of the brain. The moderate activation of NMDA receptors show increasing effect in the formation of memory, while inhibition or excessive activation of NMDAR will destroy the memory. There are some studies based on the inhibitory effect of tramadol on the NMDA and AMPA receptor which can explain the memory impairment induced by tramadol (17,33,65).

There are strong evidences suggesting that tramadol can increase the release and turnover of dopamine and serotonin after occasional administration of the drug but in chronic consumption it can deplete these neurotransmitters supplies (6,67). This fact can explain why tramadol decreased the memory following chronic treatment. Reparative responses after the chronic consumption of tramadol can demonstrate its less destructive effects on the memory.

CONCLUSION

In the preset study we showed that the administration of tramadol as an atypical opioid analgesic in a single dose or multiple doses impairs the memory in rat. The observed effects can be justified according to the unique properties of tramadol in the opioid family. It shows an inhibitory effect on a wide range of different neurotransmitters and receptors including muscarinic, NMDA, AMPA as well as some second messenger like cAMP and cGMP or its stimulatory effect on the opioid, GABA, dopamine or serotonin in the brain. More studies are requires to discover the main mechanisms involved in the inhibitory effect of tramadol on the memory.

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