Acute and Chronic Tramadol Treatment Impresses Tyrosine Kinase B (Trk-B) Receptor in the Amygdala and Nucleus Accumbens

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Abstract

Background: Misuse of opioid painkillers such as tramadol has increased in the world. These painkillers have psychological side effects such as dependence and tolerance. Moreover, the role of Tyrosine-Kinase B (Trk-B) receptor in drug dependence and reward system is not clear. The main objective of the study is to assess the effect of tramadol on the Trk-B receptors within amygdala and nucleus accumbens.

Methods: For this purpose, the male Wistar rats received different doses of tramadol (0, 5, and 10 *mg/kg*). For the assessment of the effect of acute and chronic treatment of tramadol, animals received tramadol one and 14 following days, respectively. The amygdala and nucleus accumbens (NAC) were collected, and Trk-3 protein level was quantified using Western Blotting method. The collected results were subjected into statistical analysis using SPSS software.

Results: Results showed that Trk-B level increased in the amygdala in both acute and chronic treatment. Vice-versa, tramadol treatment decrease Trk-B level in the NAC.

Conclusion: Increasing of Trk-B level in the amygdala might be related to the effect of tramadol on serotonin reuptake transporter, and it proves the anxiolytic effect of tramadol. Decreasing in the level of Trk-B in the NAC might be related to the effect of tramadol on VTA and its rewarding effect via increasing dopamine in the NAC and decreasing Trk-B level in the D1-type Medium Spiny Neurons (MSN) which enhance reward., Increasing level of Trk-B in the amygdala might be related to the anxiolytic effect of tramadol which modulates it via BDNF-Trk-B signaling pathway. More studies are needed to elucidate the effect of tramadol on BDNF-TrkB signaling pathway.

Keywords: Tyrosine kinase B receptor, Tramadol, Nucleus accumbens, Amygdala

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Introduction

Within the last year, misuse and abuse of opioid painkillers have increased in the World [1,2]. Besides the physiological effect of these analgesic drugs, they had psychological effects such as tolerance and dependence [3,4]. Tramadol is a synthetic painkiller that induced its effect via μ-Opioid Receptor (MOR) and serotonin and/or noradrenaline reuptake transporter [5]. Tramadol has two enantiomers. (+)-Tramadol is an agonist of the MOR and inhibits serotonin reuptake [6] and tramadol inhibits norepinephrine reuptake [7]. The active metabolites of tramadol are (+)-M1 and to a lesser degree (-)-M1 and (±)-N, O-didesmethyl-tramadol (M5). Neither of these compounds has an affinity with delta- and kappa-opioid receptor [8]. The neurotrophins are critical for survival and differentiation of post-mitotic neurons. The biological activity of the neurotrophins is mediated by the tyrosine kinase B (Trk-B) receptor [9,10]. Brain-derived neurotrophic factor (BDNF) binds to Trk-B and subsequently increases CREB level in neurons. So, neurons' survival, differentiation, and synaptic plasticity are practically mediated by Trk-B [11].

NAC is the essential substrate for the rewarding effect of the various drug of abuse [12]. It is proposed that NAC translates the motivational inputs into goal-directed behavior [13]. Then the Ventral Tegmental Area (VTA) and NAC express Trk-B receptors. BDNF is expressed at high levels in other regions that innervate VTA and NAC such as the amygdala, hippocampus, and frontal cortex [11]. In addition, the amygdala is an important site which is involved in addiction via conditioned-incentive learning system [14]. It is believed that projections between NAC and amygdala have an essential role in stimulus-reward association [15]. Langevin demonstrated that deep brain stimulation of the amygdala is effective in the treatment of some mental disorder such as addiction [16]. The role of TrkB linked to drug dependence and reward system is not clear. The main objective of the study is to assess the effect of tramadol on the Trk-B receptors within amygdala and nucleus accumbens.

Materials and Methods

Animals

Male Wistar Albino rats (200-220 *g*) were purchased from Pasture Institute, Tehran, Iran. The animals were maintained in the animal laboratory located at Iranian National Center for Addiction Studies (INCAS). Animals were maintained in the Plexiglas cages (3 per cage) with free access to fresh water and food at constant temperature 22±2 *°C* and 12 *hr* light/dark cycle (07:00-19:00 *hr*). The experimental procedures were in agreement with the rules of experimental animal ethics at Tehran University of Medical Sciences ethics committee.

Drug Treatment

The Tramadol HCL (Shahr Daru, Iran) was dissolved in saline (0.9%) before conducting the experiment. All purchased 36 animals were used in this study and were divided into two groups: (1) Animals that had received an acute dose of tramadol with different doses (0, 5, and 10 *mg*/*kg*) and (2) animals that received 0, 5, and 10 *mg*/*kg* of tramadol within 14 following days. Tramadol was administrated intraperitoneally (i.p). In the acute tramadol exposure, the animals were sacrificed 1 *hr* after injection.

Brain Tissue Collection

To assess expression of Trk-B in the tramadol treated animals, they were sacrificed and their amygdala and NAC were dissected immediately. The collected tissues were frozen in liquid nitrogen and had been kept at -80*°C* for conducting Western blot analysis.

Western Blotting

The level of Trk-B in the amygdala and NAC were quantified using immunoblot analysis as described previously [17]. For this purpose, proteins from both regions were extracted in Radioimmunoprecipitation Assay (RIPA) buffer. The total of 60 *μg* of proteins was loaded onto 8% polyacrylamide gel. The electrophoresis (Bio-Rad, USA) has been conducted at 120 *V* for 120 *min*. The proteins were transferred to the Polyvinylidene Fluoride (PVDF) membranes (Chemicon Millipore Co., USA). The membranes had been incubated for 60 *min* in 5% skimmed milk (Merck, Germany) to block non-specific protein binding sites. The membrane was incubated with primary antibody (Anti Trk-B receptor antibody, Abcam, 1:1000 diluted in skimmed milk) overnight at 4 *°C*. The next day, the blot was washed three times using Tris-buff-

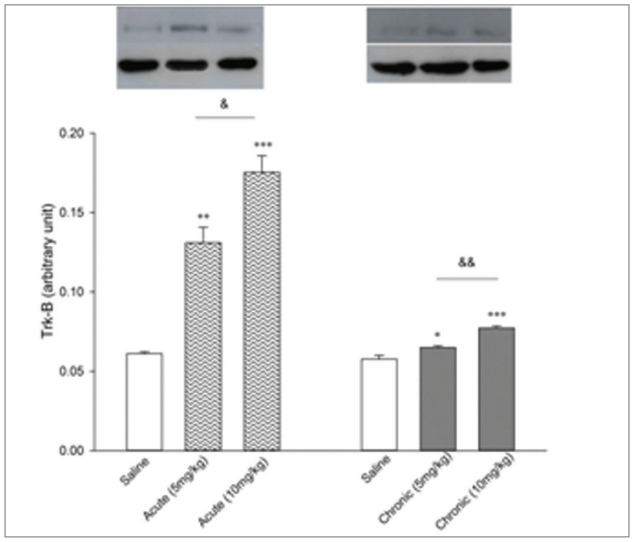

Figure 1. The protein level of Trk-B receptor in the amygdala. The density of Trk-B/β-actin in the amygdala were measured by Image J software. Bars represent Mean±SEM. (n=6). *<0.05, **<0.001, and ***<0.001 *vs*. control group.

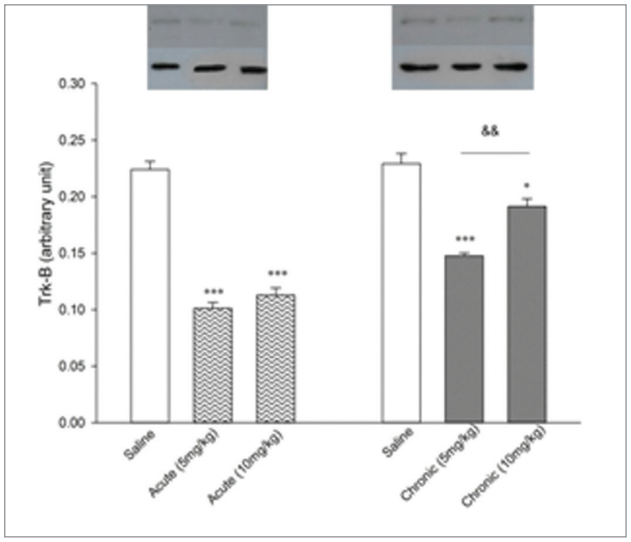

Figure 2. The protein level of Trk-B receptor in the Nucleus accumbens. The density of Trk-B/β-actin in the NAC were measured by Image J software. Bars represent Mean±SEM. (n=6). *<0.05, and ***<0.001 *vs*. control group.

ered saline and Tween 20 (TBST), then the blot was incubated with secondary antibody (Horseradish peroxidase-linked goat anti-rabbit IgG, Abcam, 1:5000) for one *hr*. After washing three times with TBST, enhanced chemiluminescence (ECL; Amersham, UK) Western blot detection system was used to detect the targeted bounds. It has been visualized by exposure to autoradiographic films for 1 to 10 *min*.

Statistical Analysis

IBM SPSS software version 21 data was used for statistical analysis. Results of western blot was quantified using densitometric scan of films with the Image J software where beta-actin (housekeeping protein) was used as endogenous control. One way ANOVA analysis and Bonferroni's post hoc analysis were performed to detect significant differences between the groups. A value lower than five present (5%) was considered as statistically significant.

Results

Trk-B increase in the amygdala during acute and chronic tramadol treatment

Figure 1 shows the Trk-B level in the amygdala of tramadol treated rats. Trk-B increases in the amygdala in the acute type of treatment [$F(2,15)=97.44$, $p<0.001$]. In addition, acute treatment of with 10 *mg*/*kg* of tramadol increases Trk-B level by 1.33 times compared with the acute administration of 5 *mg*/*kg* of tramadol ($p<0.05$).

Chronic tramadol treatment also increases Trk-B level in the amygdala [$F(2,15)=68.87$, $p<0.001$]. Also, 10 *mg*/*kg* tramadol treatment for ne 14 following days increases the level of Trk-B in the amygdala by 1.18 times in comparison with 5 *mg*/*kg* of tramadol ($p=0.005$).

The Trk-B level decrease in the NAC during acute and chronic tramadol treatment

As shown in figure 2, acute tramadol treatment at doses of 5 and 10 *mg*/*kg* decreases Trk-B level in the NAC [$F(2,15)=223.5$, $p<0.001$]. Chronic tramadol-treated rats (5 and 10 *mg*/*kg*) also showed a decrease in Trk-B level within the NAC compared with the saline-treated rats [$F(2,15)=74.48$, $p<0.001$].

Discussion

Recently, tramadol abuse is worldwide more common and this is happening in Iranian population too. Knowing the exact mechanisms which underlies tramadol action is important to develop a new treatment for tramadol abuse and poisoning. The expression pattern of Trk-B in the amygdala and NAC of the adult rat during tramadol administration have not

been analyzed in details yet. In this study, the effect of acute and chronic tramadol administration on Trk-B protein level was investigated within the NAC and amygdala using the western blotting technique.

It is known that BDNF, via its cognate receptor Trk-B, regulates the dopamine release [18]. Also, Trk-B activation can modulate dependence, sensitization, craving, relapse and other behavioral responses induced by the drug of abuse [19]. The primary results of this study showed that tramadol treatment was able to change the Trk-B level within the NAC and amygdala in both acute and chronic forms of administration. Previous data indicated that acute tramadol treatment (5 *mg/kg*) could not affect Trk-B level in the hippocampus among tested rats [20]. Moreover, during chronic (21 days) and acute tramadol administration, there was no significant change in Trk-B mRNA expression level within the PFC and hippocampus [21]. These studies were focused on the antidepressant effect of tramadol, and the regions that they selected based on the issue. As we focused on rewarding effect and abusing potential of tramadol, then we chose the NAC and amygdala regions to be tested in our study.

Chronic administration of morphine was shown to reduce K^+ conductance in the VTA dopaminergic neurons which could lead to enhancing firing rate [22,23]. Increased firing rate in VTA dopaminergic neurons could increase the dopamine level in NAC and could activate D1-type MSN [23,24]. Previous data revealed that knockout of Trk-B from D1-type MSNs increased morphine reward [25]. Therefore it can be concluded that Trk-B in the NAC D1-type MSN is essential for the rewarding effect of the drug of abuse such as opioids. Because both morphine [26] and tramadol [27] were able to induce rewarding effect via MOR, it could be suggested that tramadol led to decrease Trk-B in the NAC during chronic administration, which is in agreement with an *in vivo* study on neuroblastoma cells that showed acute dose of morphine down-regulated Trk-B [28].

Up-regulation of Trk-B is related to synaptic plasticity and survival [29]. BDNF signaling through Trk-B receptor in amygdala has an important role in the regulation of anxiety-related behavior [30]. Koponen and coworkers demonstrated that Trk-B overexpression in mice decreased the anxiety level in EPM test [31]. Basolateral Amygdala (BLA) contains two populations of neurons: (1) GABAergic interneurons and (2) projection glutamatergic pyramidal neurons. It was shown that BDNF, Trk-B and serotonin receptor expressed in both populations [32,33]. Distribution of these receptors in the BLA demonstrated that BDNF-Trk-B signaling might act on both of these cell populations to regulate the activity of the BLA.

Conclusion

It was shown that tramadol, like morphine, had an anxiolytic effect [34,35]. According to this fact that amygdala has important role in anxiety and BDNF-Trk-B signaling in the amygdala has an essential role in anxiety, we propose that tramadol anxiolytic effect mediated by serotonin is followed by BDNF-Trk-B signaling in the BLA. It means that increasing Trk-B level in the amygdala during tramadol treatment reduced anxiety. More studies are needed to elucidate the effects of long-term use of tramadol, and the impact of tramadol withdrawal on BDNF-TrkB signaling and the role of mutations, loss, or overactivation of BDNF signaling pathways on tramadol abuse.

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