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Zinc enhances the expression of morphine-induced conditioned place preference through dopaminergic and serotonergic systems

<https://doi.org/10.1515/bmc-2019-0006>

received October 21, 2018; accepted January 15, 2019.

Abstract: The antidepressant-like effects of zinc (Zn) have been documented in some animal models of depression. In addition, antidepressants may reduce the abuse potential of opioids by affecting their rewarding effect. Hence, this study was performed to investigate the effect of Zn on the expression of morphine-induced conditioned place preference (CPP) in male rats. We used an unbiased CPP paradigm for investigating the effect of Zn. The intraperitoneal (i.p.) and intracerebroventricular (i.c.v.) administrations of Zn (5-20 mg/kg, i.p., and 10 nmol/rat, respectively) with or without morphine did not induce conditioned place aversion (CPA) or CPP during acquisition phase. However, the same i.p. and i.c.v. administrations of Zn induced morphine-like CPP in the expression phase. Pre-treatment with dopamine receptor antagonists (SCH23390, sulpiride, and haloperidol) and serotonin receptor antagonists (WAY100135, ketanserin, and ondansetron) reversed the enhancement effect of Zn on the expression of morphine-induced CPP (especially 20mg/kg, i.p. and 10 nmol/rat, i.c.v.). These findings suggest that acute i.p. and i.c.v. administration of Zn might enhance the rewarding properties of morphine through involvement with dopaminergic and serotonergic neuronal systems.

Keywords: Morphine; Zinc sulfate; Conditioned place preference; Reward; Expression; Rat.

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Introduction

Endogenous opioids and opioid analgesics, i.e. morphine, have been of interest because they have analgesic and psychologically reinforcing effects which can involve in abuse [1]. Some researchers reported that morphine has positive reinforcement through activation of μ receptor which facilitates dopamine (DA) release [2-4]. Moreover, the drugs that increase central serotonin (5-HT) levels, such as serotonin agonists and selective serotonin reuptake inhibitors (SSRIs), diminish the intake of rewarding stimuli, including drugs of abuse, suggesting that these drugs reduce reinforcement [5, 6]. For instance, electrophysiological investigations have documented that SSRIs (e.g. fluoxetine) decrease the spontaneous function of DA neurons in the ventral tegmental area (VTA), showing that 5-HT involves in this region [7]. On the other hand, 5-HT indirectly stimulates DA release [6]. Therefore, serotonergic and dopaminergic systems modulate in the regulation of the rewarding processing.

The conditioned place preference (CPP) paradigm is widely applied for investigating the reinforcing effects of natural and pharmacological stimuli, such as abusing drugs, as this method can identify reinforcing effects [8].

Zinc (Zn), an essential trace element for brain activity, is involved in specific areas of the brain including the hippocampus, amygdala, and cortex [9]. Zn interferes in neuronal excitability [10] and synaptic plasticity [11]. Furthermore, Zn is as a signaling molecule [12]. Paluma et al. [13] showed that Zn has a protective action through the dopaminergic system against cadmium injuries, suggesting that Zn has relations with dopaminergic system. In support of this view, a study shows that after the prenatal exposure to zinc oxide nanoparticles, the turnover of DA increased in the prefrontal cortex, neostriatum, nucleus accumbens (NAC) and the amygdala. Meanwhile, the level of DA was increased in the hippocampus of mouse offspring during this period [14]. Apart from the dopaminergic system, Zn may be directly involved in the function of the serotonergic system [15]. In this regard, Szewczyk et al.

[16] revealed that Zn produced antidepressant-like effects via modulation by the serotonergic system in the forced swimming test (FST). The antidepressant-like effect of Zn was blocked by pre-treatment with serotonin synthesis inhibitor, *p*-chlorophenylalanine (pCPA), WAY100635 (5HT-1A receptor antagonist), and ritanserin (5HT-2A/C receptor antagonist). Moreover, Cunha et al. [17] reported the synergistic effects between Zn and dopaminergic or serotonergic neural systems in animal models of depression.

Apart from these findings, a study showed that antidepressants may reduce the abuse potential of opioids by affecting their rewarding effect or improving a depressed mood resulting from opioid withdrawal [18]. Consistent with this theory, different investigations have shown that herbal (such as hypericin) or synthetic antidepressants (such as fluvoxamine, sertraline or nortriptyline) enhanced the expression of morphine-induced CPP. On the other hand, they could stimulate the brain reward function, suggesting it might be involved in their therapeutic efficacy in opioid addiction [6, 19 - 21].

Considering this background as well as no evidence of the effect of Zn on morphine conditioning and reward, the present study sought to investigate the effect of Zn on the expression of morphine-induced CPP. Moreover, we assessed the possible involvement of dopaminergic and serotonergic systems on the effect of Zn and its effect on the expression of morphine-induced CPP in male rats.

Material and Methods

Animals

One hundred fifty-six male Wistar rats (Urmia University of Medical Science, Urmia, Iran), weighing 200-250 g, were used at the start of the experiment. The rats were grouped in cages with free access to standard rat food (supplied by Razi Vaccine & Serum Research Institute, Karaj, Iran) and tap water and they were maintained with a 12-h light/dark cycle, in a temperature (20-22 C) and humidity-controlled room (approximately 60%). The time distance between delivery of the animals and trial start was at least five days. Each experimental group consisted of 6 animals. The trial was done between 8:00 a.m. and 5:00 p.m. Each rat was used only once.

Ethical approval: The research related to animals use has been complied with all the relevant national regulations and institutional policies for the care and use of animals. This study was approved by the ethics

committee of Urmia University of Medical Science (code; UUMS-REC.2049 date; Aug 2017).

Drugs

We prepared drugs and chemical agents as follows: morphine sulfate (Temad Co, Tehran, Iran), Zn sulfate (Alhavi Pharmaceutical Co, Tehran, Iran), SCH23390 (Sigma-Aldrich, St. Louis, MO, USA), Sulpiride (Sigma-Aldrich, St. Louis, MO, USA), Haloperidol (Caspian Tamin, Tehran, Iran), WAY100135 (Sigma-Aldrich, St. Louis, MO, USA), ketanserin (Sigma Aldrich, St. Louis, MO, USA), and ondansetron hydrochloride (Tehran Chemie Pharmaceutical Co, Tehran, Iran). The drugs were dissolved in normal saline (Shahid Ghazi Pharmaceutical Co, Tehran, Iran) and injected intraperitoneally and intracerebroventricularly (i.c.v.) at constant volume of 1 ml/kg and 0.5 μ l per rat, respectively. Also, all drugs were prepared immediately before use. All drug doses and the administration schedule were chosen based on previous literature data that confirm the efficacy of the above-mentioned protocols [6, 16, 22, 23].

Apparatus

A three-compartment place preference apparatus was made of Plexiglas, measuring 88×36×34 cm, consisting of two main compartments measuring 39×36×34 cm, one having grey sides with a smooth grey plate, the other with black and white stripes (2 cm wide) and with a smooth white plate. The third compartment was composed of a white central platform measuring 10×36×34 cm and rose by 2 cm, which separated the two main sections. At conditioning phase, compartments were isolated using guillotine doors [6, 22].

Surgery and intracerebroventricular injection

The animals were anesthetized by i.p. administration of xylazine hydrochloride (5 mg/kg, Alfasan, Netherlands) and ketamine hydrochloride (80 mg/kg, Alfasan, Netherlands), and placed in a stereotaxic apparatus (Stoelting model 51600, IL, USA). An i.c.v. cannula, internally protected by a stylus, was embedded in the lateral ventricle on basis of parameters from the bregma alignment: AP=-0.5 mm caudal to bregma, Lat=1.6 mm lateral to midline, DV=4.2 mm ventral from the skull surface [24]. Finally, the assembly of the cannula was anchored

to the skull with dental acrylic and 4 small stainless steel screws. After the surgery, the rats were individually placed in transparent Plexiglas cages for seven days until recovery time. Five microliter microsyringes were applied to inject the drugs. A polyethylene tube was used to attach the injection cannula to the microsyringe. The 0.5 μ l of a drug was slowly delivered over a 30 second period. At the end of the experiment, the position of the cannula was histologically identified with the administration of 2.5 ml of blue dye.

Conditioned place preference procedure

Like our previous studies [6, 25], unbiased CPP paradigm took place over six continuous days and it was created from three separate phases:

Pre-conditioning phase

On the first day of the trial, the guillotine doors were opened and each animal was individually positioned into the middle part of the apparatus for 10 min with free access to all compartments. The time spent in each compartment was measured to investigate the least preferred side for each rat [6, 22, 25].

Conditioning phase

This phase was conducted over 4 days and the guillotine doors were closed. The rats received drugs on day 1 and day 3 and confined for 30 minutes to their least preferred (drug-paired) compartment. During days 2 and 4, the rats were given saline and confined for 30 minutes with their preferred (saline-paired) compartment.

Post-conditioning phase

This phase was done over 6 days of the trial (1 day after the last conditioning session). Same as the pre-conditioning phase the guillotine doors were open. The rats had free access to all compartments for 10 minutes and morphine was not administered at this phase [6, 22, 25]. At all phases, animals were tested during the time period 08:00-15:00 each day. The time spent in the least preferred (drug-paired) compartment was recorded by a chronometer (Citizen, Japan). Rats that spent 6.5 min (65%) or more of the total test time in one of the cue-compartments were

excluded from the experiment in order to maintain an unbiased protocol.

Experimental design

Dose-response effects of CPP induced by morphine sulfate

Based on our previous studies and in accordance with other findings [6, 22, 25], 5 mg/kg of morphine (82.09 ± 3.664 s) [One-way analysis of variance (ANOVA); $F(4, 25) = 140.1, p < 0.001$] was chosen as the effective dose for the conditioning sections. During the first and third day of the conditioning phase, the rats were treated with morphine and positioned in the least preferred side of apparatus for 30 minutes. During the second and fourth days of the conditioning phase, the rats were treated with saline (1 ml/kg, i.p.) and positioned on the preferred side of apparatus for 30 minutes. The rats treated with saline in both sides served as control groups.

Effects of Zn on the acquisition (devolement) of morphine-induced CPP

In the first set of the experiment and during the conditioning phase, 30 minutes prior to the placement of animals in the least preferred compartment, the animals separately received three i.p. (5, 10, and 20 mg/kg) an i.c.v. (10 nmol/rat) dose of Zn alone (without morphine). The control group received saline (10 ml/kg, i.p.) in place of Zn.

In the second set of the experiment and during the conditioning phase, 30 minutes prior to the placement of animals in the least preferred compartment, the animals separately received the same dose of Zn with morphine (5 mg/kg, i.p.). The control group received saline (10 ml/kg, i.p.), 30 minutes prior to the conditioning of the animals with morphine (5 mg/kg, i.p.).

Effects of Zn on the expression of morphine-induced CPP

In order to investigate the effect of Zn on the expression of morphine-induced CPP, all animals received morphine (5 mg/kg, i.p.) for conditioning, as previously described. However, in the post-conditioning or on the test day, the rats separately received three i.p. (5, 10, and 20 mg/

kg) an i.c.v. (10 nmol/rat) doses of Zn. The CPP test was performed at both 30 minutes and 15 minutes after i.p. and i.c.v administration of the drugs, respectively.

Examination of the mechanism of the enhancement effect of Zn on the expression of morphine-induced CPP

Involvement of the dopaminergic system on the effect of Zn to enhance morphine-induced CPP

For examination of the possible contribution of the dopaminergic system on the enhancement effects of Zn on the expression of morphine-induced CPP, the animals were conditioned with morphine under the schedule described above. Then, 30 minutes and 15 minutes prior to the administration of Zn (20 mg/kg, i.p. and 10 nmol/rat, i.c.v., respectively) different groups of animals received SCH23390 (0.05 mg/kg, i.p., a dopamine D1 receptor antagonist), sulpiride (5 mg/kg, i.p., a dopamine D2 receptor antagonist), and haloperidol (0.2 mg/kg, i.p., *non-selective dopamine* receptor antagonist). Finally, the CPP test was performed 30 minutes and 15 minutes after i.p. and i.c.v administration of the drugs, respectively.

Involvement of serotonergic system in the effect of Zn enhance morphine-induced CPP

To examination the possible contribution of the serotonergic system on the enhancement effects of Zn on the expression of morphine-induced CPP, the same protocol from the previous experiment was performed, but instead, 30 minutes and 15 minutes prior to the administration of Zn (20mg/kg, i.p. and 10 nmol/rat, i.c.v., respectively) different groups of rats received WAY100135 (10 mg/kg, i.p., a selective 5-HT_{1A} receptor antagonist) ketanserin (5 mg/kg, i.p., a selective 5HT_{2A/C} receptor antagonist) and ondansetron (5 mg/kg, i.p., 5-HT₃ antagonist). Finally, the CPP test was performed 30 minutes and 15 minutes after i.p. and i.c.v administration of the drugs, respectively.

Statistical analysis

In the CPP test, the values (means \pm the standard error of the mean (SEM)) are presented as the least amount of time (s) spent in a compartment, before and after conditioning, following one-way ANOVA, Tukey's *post-hoc* test was used to evaluating the significance of the drugs and chemical

agents. A value of $p < 0.05$ was considered statistically significant. In the present study, all statistical analyses were also performed using Prism with version 7 (GraphPad Software, Inc., San Diego, CA, USA).

Results

Dose-response curve for morphine-induced place conditioning

Based on our previous studies and in accordance with others, 5 mg/kg of morphine sulfate achieved the maximum response, so we used this dose for subsequent experiments. The control treatment, saline, did not show any preference for the compartments.

Effect of Zn on the acquisition (development) of morphine-induced CPP

The effect of i.p. (5-20 mg/kg) or i.c.v. (10 nmol/rat) administrations of Zn alone (without morphine) is presented in Figure 1. In addition, the effect of the same doses of Zn with morphine (5 mg/kg) during the conditioning phases is presented in Figure 2. There was no significant interaction between Zn and morphine on conditioned place aversion (CPA) or CPP [One-way ANOVA; $F(4, 25) = 6.861, p > 0.05$ and $F(4, 25) = 2.137, p > 0.05$, respectively].

Effect of Zn on the expression of morphine-induced CPP

The effect of three i.p. (5-20 mg/kg) and an i.c.v. (10 nmol/rat) doses of Zn with morphine (5 mg/kg) on the expression of morphine-induced CPP is presented in Figure 3. Statistical analysis showed a significant interaction between morphine and Zn on CPP during the expression phase [One-way ANOVA; $F(4, 25) = 13.45, P < 0.001$]. Our result reveals that Zn (20 mg/kg, i.p. and 10 nmol/rat, i.c.v., respectively) significantly enhanced the expression of the morphine induced-CPP. Hence, these doses were chosen for subsequent experiments.

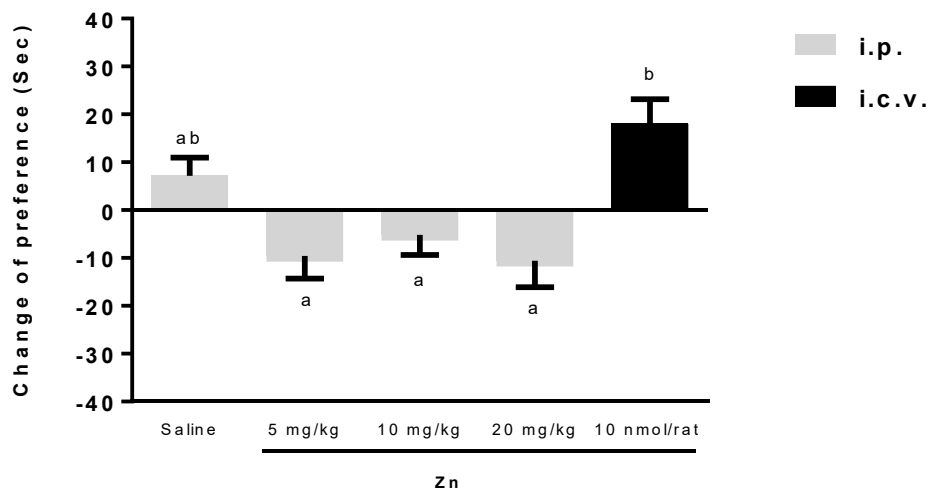


Figure 1. Effects of Zn without morphine on the acquisition (development) of CPP. Zn was given i.p. (5, 10 and 20 mg/kg) and i.c.v. (10 nmol/rat) separately in the conditioning sections in least preferred compartments. Each point represents the mean± SEM of six rats. Different letters indicate significant differences between groups ($p < 0.05$) (Tukey's *post-hoc* test). Zn=Zinc.

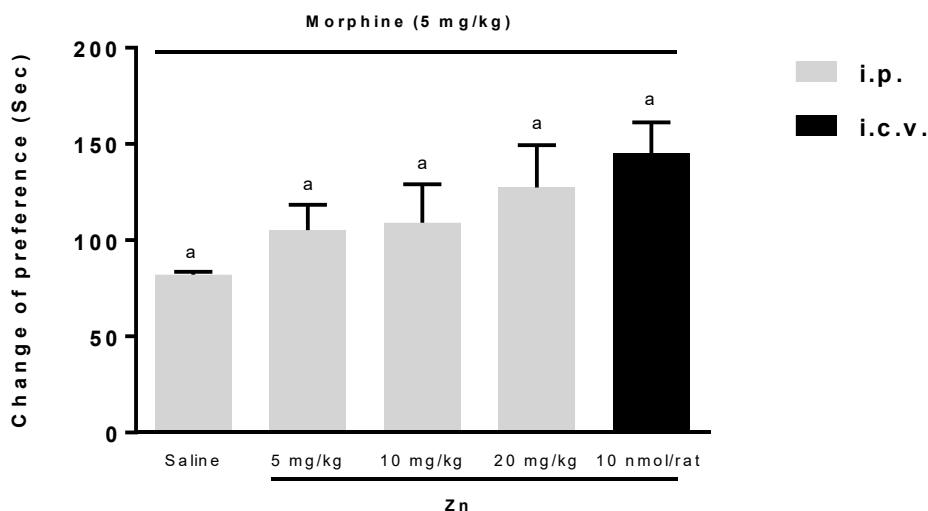


Figure 2. Effects of Zn with morphine on the acquisition (development) of CPP. Zn was given i.p. (5, 10 and 20 mg/kg) and i.c.v. (10 nmol/rat) separately with morphine (5mg/kg) in the conditioning sections in least preferred compartments. Each point represents the mean± SEM of six rats. Zn=Zinc.

Elucidation of involvement mechanisms in the expression of morphine-induced CPP

Examination of the involvement of Zn in the dopaminergic system

The effects of the pre-treatment of rats with dopamine receptor antagonists (SCH23390, sulpiride, and haloperidol) are presented in Figures 4-6. Our findings

suggested that pre-treatment with SCH23390 [One-way ANOVA; $F(2,15) = 203.4, p < 0.001$], sulpiride [One-way ANOVA; $F(2,15) = 253.5, p < 0.001$], and haloperidol [One-way ANOVA; $F(2,15) = 1015, p < 0.001$] reversed the enhancement effect of Zn on the expression of morphine-induced CPP (10-20 mg/kg, i.p. and 10 nmol/rat, i.c.v.).

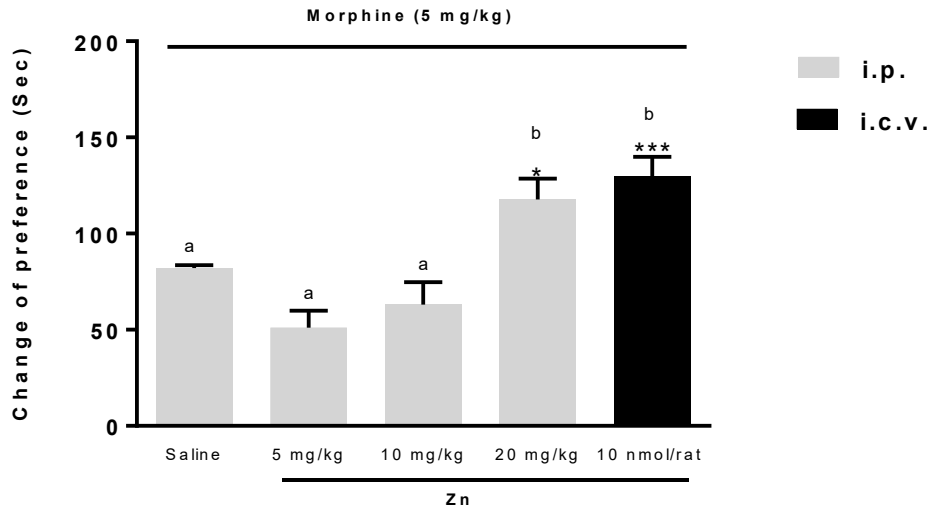


Figure 3. Effect of Zn on the expression of morphine-induced CPP. Zn was given i.p. (5, 10 and 20 mg/kg) and i.c.v. (10 nmol/rat) separately in the post-conditioning sections. Each point represents the mean \pm SEM of six rats. * and ***Presents significant between saline (10 ml/kg, i.p.) group at $p < 0.05$ and $p < 0.001$, respectively. Different letters indicate significant differences between groups ($p < 0.05$) (Tukey's *post-hoc* test). Zn=Zinc.

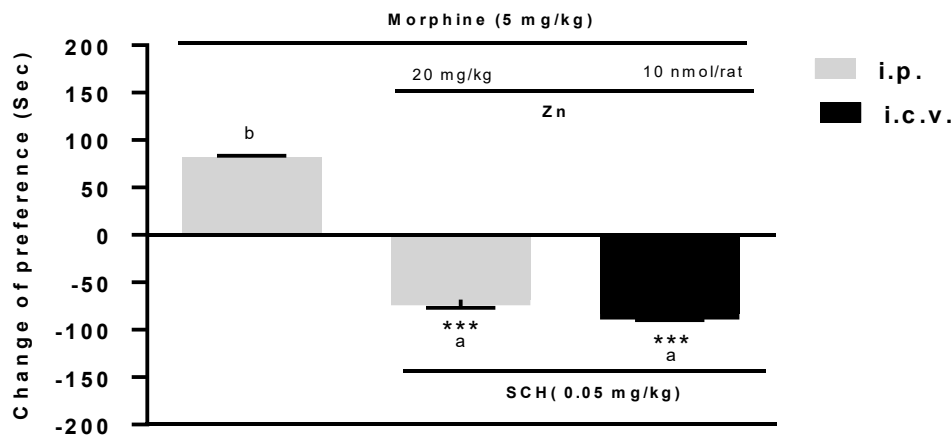


Figure 4. Effect of SCH23390 on the enhancement effect of Zn on the expression of morphine-induced CPP. Zn was given i.p. (20 mg/kg) and i.c.v. (10 nmol/rat) separately after the SCH23390 treatment in the post-conditioning sections. Each point represents the mean \pm SEM of six rats. *** Presents significant between saline (10 ml/kg, i.p.) group at $P < 0.001$. Different letters indicate significant differences between groups ($p < 0.05$) (Tukey's *post-hoc* test). Zn=Zinc, and SCH=SCH23390.

Examination of the involvement of Zn with the serotonergic system

The effects of the pre-treatment of animals with serotonin receptor antagonists (WAY100135, ketanserin, and ondansetron) are shown in Figures 7-9. One-way ANOVA analysis indicated that these drugs reversed the

enhancement effect of Zn on the expression of morphine-induced CPP [(20 mg/kg, i.p. and 10 nmol/rat, i.c.v.); WAY100135, $F(2, 15) = 100.3$, $p < 0.001$; ketanserin, $F(2, 15) = 133.8$, $p < 0.001$; ondansetron, $F(2, 15) = 463.9$, $p < 0.001$].

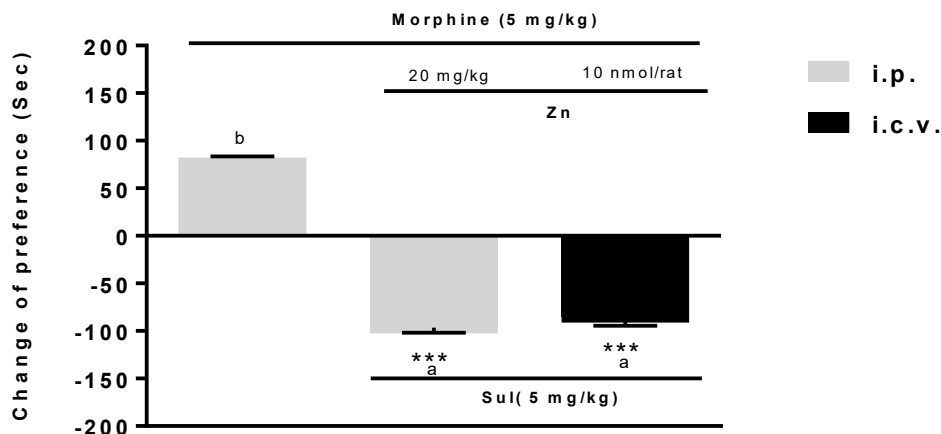


Figure 5. Effect of sulphiride on the enhancement effect of Zn on the expression of morphine-induced CPP. Zn was given i.p. (20 mg/kg) and i.c.v. (10 nmol/rat) separately after the sulphiride treatment in the post-conditioning sections. Each point represents the mean \pm SEM of six rats. *** Presents significant between saline (10 ml/kg, i.p.) group at $P < 0.001$. Different letters indicate significant differences between groups ($p < 0.05$) (Tukey's *post-hoc* test). Zn=Zinc, and Sul= sulphiride.

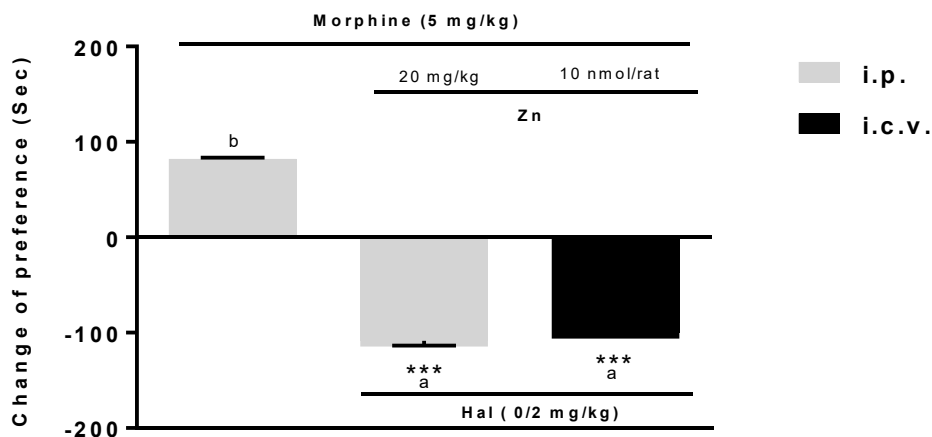


Figure 6. Effect of haloperidol on the enhancement effect of Zn on the expression of morphine-induced CPP. Zn was given i.p. (20 mg/kg) and i.c.v. (10 nmol/rat) separately after the haloperidol treatment in the post-conditioning sections. Each point represents the mean \pm SEM of six rats. *** Presents significant between saline (10 ml/kg, i.p.) group at $P < 0.001$. Different letters indicate significant differences between groups ($p < 0.05$) (Tukey's *post-hoc* test). Zn=Zinc and Hal= haloperidol.

Discussion

The current study examined the effect of Zn on the expression of morphine-induced CPP in rats. Our observations demonstrated, for the first time, that Zn did not produce any significant CPP or CPA when it was applied with or without morphine during the acquisition phase. These findings suggest that Zn is not addictive when administered alone and it may have therapeutic potential for opioid addiction. Previous studies relieved

that Zn is relatively harmless and only high doses have toxic effects (such as lethargy, focal neurological deficits, nausea, diarrhea, and elevated the risk of prostate cancer) [26]. However, the oral LD_{50} of Zn is 3 g/kg body weight [27]. Furthermore, a study showed that the exposure to 12 g of metallic Zn after three days produced lethargy and focal neurological deficits in the brain [28]. Despite this, two protective factors, including systemic homeostasis and efficient cellular regulatory mechanisms, prevent the uptake of cytotoxic doses of exogenous Zn. Nevertheless,

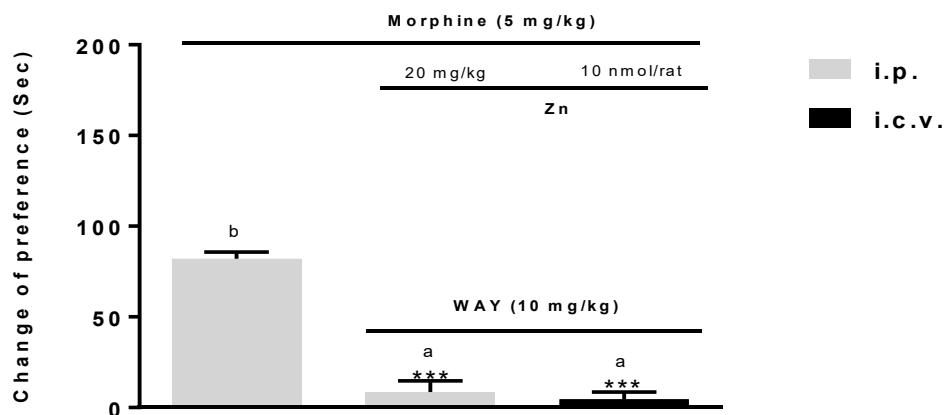


Figure 7. Effect of WAY100135 on the enhancement effect of Zn on the expression of morphine-induced CPP. Zn was given i.p. (20 mg/kg) and i.c.v. (10 nmol/rat) separately after the WAY100135 treatment in the post-conditioning sections. Each point represents the mean \pm SEM of six rats. *** Presents significant between saline (10 ml/kg, i.p.) group at $p < 0.001$. Different letters indicate significant differences between groups ($p < 0.05$) (Tukey's *post-hoc* test). Zn=Zinc, and WAY= WAY100135.

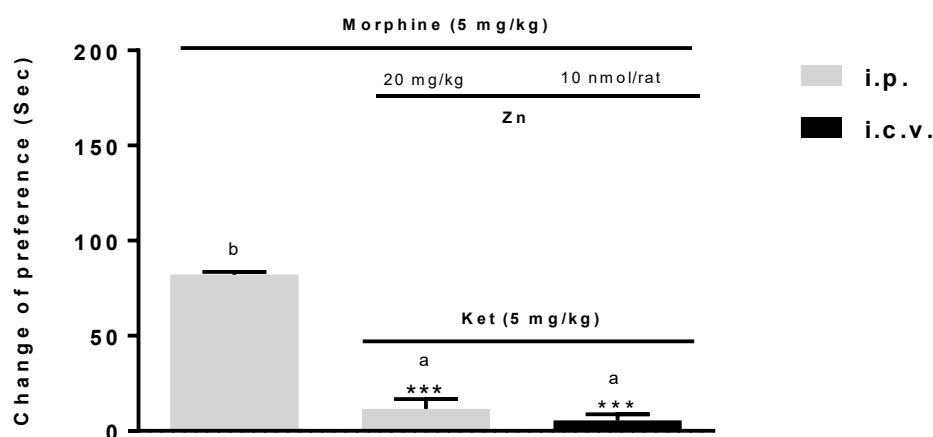


Figure 8. Effect of Ketanserin on the enhancement effect of Zn on the expression of morphine-induced CPP. Zn was given i.p. (20 mg/kg) and i.c.v. (10 nmol/rat) separately after the ketanserin treatment in the post-conditioning sections. Each point represents the mean \pm SEM of six rats. *** Presents significant between saline (10 ml/kg, IP) group at $p < 0.001$. Different letters indicate significant differences between groups ($p < 0.05$) (Tukey's *post-hoc* test). Zn=Zinc, and Ket= Ketanserin.

in the present study, all of the applied doses were significantly below the Zn LD_{50} or toxic doses [26].

In addition, our results exhibited that acute peripheral and especially central (i.c.v.) administrations of Zn with morphine enhances morphine-induced CPP during the expression phase. In line with other study [29], our data indicated that the Zn can pass through the blood-brain barrier (BBB) and affect the nervous system. On the other hand, Zn can transport through the BBB and increase morphine-induced CPP. As the results indicated, high doses of Zn (especially 20 mg/kg, i.p.) created CPP and interacted with serotonergic and dopaminergic systems.

In accordance with our findings, other studies have shown that animals receiving saline in both compartments did not show an overall preference for CPP [6, 22].

As Zn has blocking or reducing activities of dopamine uptake [30], a series of experiments were performed with dopamine receptor antagonists (SCH23390, sulpiride, and haloperidol), in order to observe the involvement of dopaminergic mechanisms in the elicited response by Zn. However, our results indicate that dopamine receptors are involved in the enhancement effect of Zn on the expression of morphine-induced CPP (20 mg/kg, i.p., and 10 nmol/rat, i.c.v.) because the effect of Zn on

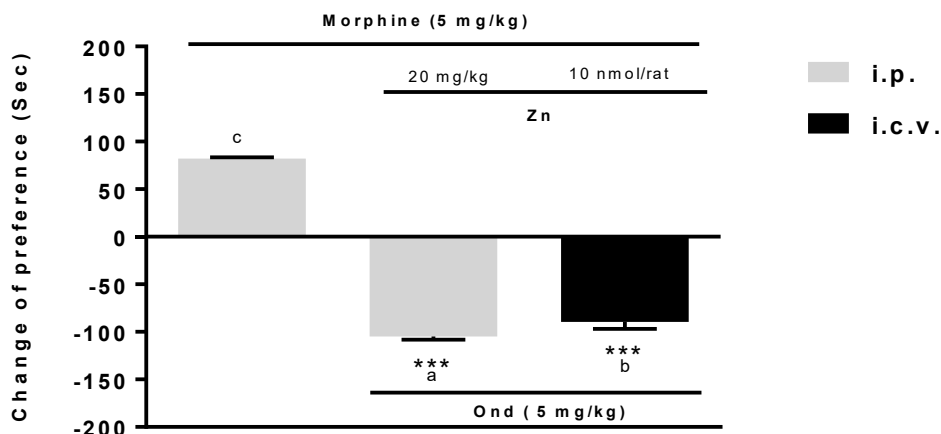


Figure 9. Effect of ondansetron on the enhancement effect of Zn on the expression of morphine-induced CPP. Zn was given i.p. (20 mg/kg) and i.c.v. (10 nmol/rat) separately after the ondansetron in the post-conditioning sections. Each point represents the mean \pm SEM of six rats. *** Presents significant between saline (10 ml/kg, i.p.) group at $P < 0.001$. Different letters indicate significant differences between groups ($p < 0.05$) (Tukey's *post-hoc* test). Zn=Zinc, and Ond=ondansetron.

morphine-induced CPP was reversed by pre-treatment with SCH23390 (a dopamine D1 receptor antagonist), haloperidol (*non-selective dopamine* receptor antagonist), and sulpiride (a dopamine D2 receptor antagonist). On the other hand, the activation of dopaminergic system is essential for rewarding properties. A study has shown that endogenous opioid systems influence DA release in the mesolimbic system [31]. However, a high number of medium spiny neurons are present in the striatum [32] and that these neurons within the nucleus accumbens (NAC) contain dopaminergic synapses, which are the important output neurons of the mesolimbic DA system and may potentiate reward properties [33]. It is well shown that different types of drugs of abuse activate the mesolimbic dopamine system in different ways [2-4]. However, administration of DA in the NAC directly caused an increase in response for reward properties [34]. Also, a study has shown that DA and 5-HT are related, in so that both regulate reward properties. Although they may act by different routes, a balance between DA and 5-HT influences reward properties [35]. Sasaki-Adams and Kelley [34] demonstrated that acute administration of fluoxetine increased the release of 5-HT. In addition they showed that it may induce dopamine release in the NAC, showing an increase in response to the conditioned reinforcement. Considering given background, a major relation between dopaminergic system and rewarding system can be made. It seems Zn acts through the dopaminergic system, because its effect reversed by DA receptor antagonists. On the other hand, Cunha et al. [17] reported that Zn, as an antidepressant, has synergistic effects with dopaminergic

systems. Ciubotariu et al. [23] documented that deficiency in Zn levels influences the main neurotransmitters for development and maintaining the addiction, i.e. DA, etc. Zn seems to be a moderator in the dopaminergic system since it prevents DA transport through the neuronal DA transporter [36].

Our findings showed that pre-treatment with 5-HT receptor antagonists (WAY100135, ketanserin, and ondansetron) reversed the role of Zn to enhance the expression of morphine-induced CPP (20 mg/kg, i.p., and 10 nmol/rat, i.c.v.); showing that Zn is involved with the serotonergic system. Similar to the dopaminergic system, the serotonergic system modulates rewarding properties. On the other hand, studies showed that activation of 5HT2 receptors of the posterior shell of NAC stimulates DA release. However, 5-HT is an efficient promoter for DA release [37]. Thus, an increase in brain 5-HT may decrease some aspects of morphine withdrawal related with reduction of DA, which encourages the reward pathway [3]. These studies confirmed the relation between the serotonergic system and the reward system. Therefore, Zn may act through the serotonergic system since its effect was reversed by 5-HT receptor antagonists. On the other hand, Barrondo and Salles [38] suggested modulatory effects of Zn on the presynaptic 5HT1A and serotonergic neurotransmission. The effect of Zn, as an antidepressant, was reversed by pre-treatment with inhibitors of 5-HT synthesis, i.e. 5HT-2A/C antagonist and 5HT1A antagonist [16]. In line with our findings, some researchers have indicated that the antidepressant-like effects of Zn was modulated through the serotonergic system [39]. Parallel

to our observation, AL Amry and SSC-Psych [40] indicated that Zn indirectly releases 5HT and activates 5HT receptors. In other studies, Zn in combination with SSRIs showed synergistic antidepressant-like effect [15, 41]. On the other hand, earlier studies have shown a positive correlation between fluoxetine and Zn [42]. In support of this view, a study showed that fluoxetine enhances the rewarding effect of morphine in CPP paradigm [43] or modulates morphine-induced behavioral sensitization [44]. There was a significant relationship between fluoxetine and the dopaminergic system for induction of transmission [45]. Nowak et al. [46] showed Zn, in combination with fluoxetine, is effective for treatment of patients with severity depression. In animal studies, co-administration of sub-effective doses of antidepressants, fluoxetine, and very low doses of Zn was efficient compared to single form [16, 17], showing that Zn has a synergistic effect with fluoxetine.

Conclusion

Our results, for the first time, showed that acute peripheral and central administration of Zn enhances the expression of morphine-induced CPP and it may be associated with the dopaminergic and serotonergic mechanism(s). Moreover, this effect is reversed by SCH23390, haloperidol, sulpiride, WAY100135, ketanserin, and ondansetron. Hence, the enhancement effect of Zn on the expression of morphine-induced CPP, at least in part, via modulation of DA, 5-HT_{1A}, 5HT_{2A/C} and 5-HT₃ receptors, respectively. Also, acute i.p. administration of Zn could show better interactions. However, this study showed a novel association between morphine-induced CPP and Zn.

Conflict of interest: Authors state no conflict of interest.

Acknowledgments: This study was financially supported by a research project (No.51035911201001) from the Urmia Branch, Islamic Azad University, Urmia, Iran. But, we appreciate of Dr. Mehdi Zavari the Vice President of Research Islamic Azad University of Urmia Branch for supporting our research project.

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