Intervention of the Gamma-Aminobutyric Acid Type B Receptors of the Amygdala Central Nucleus on the Sensitivity of the Morphine-Induced Conditionally Preferred Location in Wistar Female Rats

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Original Article

Abstract

Background: The amygdala is one of the nerve centers involved in drug reward. It is suggested that the central nucleus of the amygdala (CeA) is involved in morphine dependency. The CeA gamma-aminobutyric acid-ergic (GABAergic) system is a mediator of morphine rewarding effects. In this research, the effects of stimulation or inhibition of CeA GABA type B ($GABA_B$) receptors on sensitization acquisition to morphine-induced reward was evaluated in Wistar female rats using conditioned place preferential (CPP) method.

Methods: Wistar female rats provided by Shahid Beheshti University, Tehran, Iran, were allocated into 17 groups including 7 groups of determining morphine dose-response, 2 groups of sensitivity and control, and 8 groups of different doses of agonists and antagonists in the acquisition stage (n = 7 in each group). Various quantities of morphine (0.5, 1, 2.5, 5, 7.5, 10 mg/kg of animal weight) were used to determine the effective and neutral doses of morphine. After 5 days from the start of the surgery, sensitization was induced. After the end of the sensitization period, CPP was conducted. Baclofen and CGP35348, as an agonist and antagonist of $GABA_B$ respectively, with the dose of 1.5, 6 and 12 μg/rat were inserted to the CeA, ten minutes before taking morphine.

Findings: Administration of baclofen had no significant effect on the acquisition of morphine sensitization. In contrast, injection of CGP35348 reduced the sensitivity to morphine.

Conclusion: GABA receptors can be effective in reducing morphine tendency by specific receptors, so these sites can be important therapeutic targets in counteracting the effects of drug abuse.

Keywords: Morphine; Baclofen; Rats; Amygdala

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Introduction

The abuse of opioids such as morphine is a serious problem that affects health, social life, and welfare of all communities.[1,2] Frequent consumption of morphine, along with behavioral changes, as well as rewarding[3] and withdrawal symptoms lead to morphine sensitization.[4] Morphine sensitization is a form of long-lasting use of morphine which increases the possibility of relapse. Different forms of sensitization, like behavioral withdrawal, may intensify the process of morphine sensitization. Behavioral sensitization is usually associated with motor responses and can be accessed through conditioned place preferential (CPP).[5,6] In the CPP paradigm, sensitization is characterized with increasing time spent in the drug-paired chamber.[7,8]

Acquiring is one of the basic steps in drug sensitivity. This stage consists of immediate neurologic events with unpleasant behavior symptoms.[9]

The importance of the central nucleus of the amygdala (CeA) gamma-aminobutyric acid-ergic (GABAergic) system in response to morphine has been proven with strong evidence.[10] By using an addictive drug such as morphine, the mesolimbic center becomes activated. GABA receptors with inhibitory function adjust the release of dopamine transmitter, which is one of the rewarding agents mediating the abuse effects of morphine, from mesolimbic. Thus, in morphine response, the activation ratio of dopamine and GABA receptors is very important.[8] Also, ventral tegmental is one of the mesolimbic rewarding areas in which the presence of a large number of GABA type B ($GABA_B$) receptors has been demonstrated. Baclofen injection in this area causes dopamine release inhibition from other mesolimbic regions, such as nucleus accumbens. By inhibiting the release of dopamine, the positive response to morphine is also reduced.[11,12] Considering the relationship between the CeA and the nucleus accumbens,[10] application of baclofen in the CeA (chronic baclofen treatment) decreased the morphine-induced release of dopamine.[13]

Most of the researches on rewards and attempts are generally done on the male gender, in order to identify and control rewards.[14] Furthermore, little is known about the role of inhibitory receptors, such as $GABA_B$, in the tendency and sensitivity to morphine. Given the fact that a large percentage of morphine addicts in the society are female and there are differences between males and females in terms of the drug response,[15] the purpose of this research was to clarify the importance of stimulating and inhibiting the core of amygdala on the morphine tendency in females. Our conducted experiments could be substantially important in further investigations on morphine effects in females and search for new therapeutic strategies for morphine-addicted female patients.

Methods

The present study was an experimental research. Each group consisted of 7 Wistar female mice in the weight range of 250 to 275 gram. Animals were exposed to appropriate light and food conditions. The allocation of animals into cages was completely randomized. Ethics were considered in all aspects.

Drugs used in this study including morphine combined with sulfate (prepared in Iran), baclofen as a stimulant, CGP35348 with inhibitory function (made in Switzerland), anesthetic drugs including Ketamine (70 mg/kg of animal weight) and Xylazine (10 mg/kg of animal weight) were injected intraperitoneal. The medications were dissolved in normal saline. Various quantities of subcutaneous morphine (0.5, 1, 2.5, 5, 7.5, 10 mg/kg of animal weight) were injected to animals. Also, the inhibitory and stimulating drugs were injected into the CeA at 3 doses of 1.5, 6 and 12 µg/rat.[7,8] To ensure that the drug is transferred to the desired core, the needle tip remained within the core for one minute after injection. Next, CPP measurement was conducted after two minutes of animals rest.[7]

By using the stereotactic device and dental cement, two cannulas with 23 gauge thickness were fixed at the target core**.** Stereotactic dimensions for the CeA were based on Paxinos and Watson color atlas information which are -3.3 mm for incisor bar, -7.8 mm dorsal-abdominal, -2.12 mm anterior-posterior and ± 4.1 mm middle-lateral.[16] After the surgery, the animals were recovered for a week.

The CPP device used in this experiment consisted of two parts, with the dimensions of 30 × 60 × 30 centimeters.[15] Also, two sides of the box had equal dimensions, but the signs of the two parts and symptoms of smells were different.

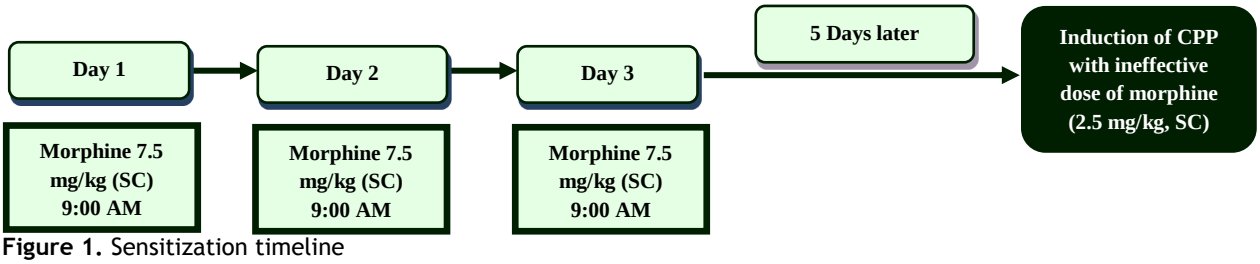

Figure 1. Sensitization timeline
SC: Subcutaneous; CPP: Conditioned place preferential

Besides, animals did not have a preference on either side of the box in the preconditioning period. This supports our unbiased conditioned place preference paradigm.

Five days after the surgery and cannulation, sensitization was induced in rats through receiving 7.5 doses of morphine for 3 consecutive days. Five days later, CPP was done with 2.5 doses of morphine. Baclofen or CGP35348 were injected inward the CeA, ten minutes before each morphine taking in the first, second and third days of sensitization.[8] Sensitization timeline is shown in figure 1.

CPP included a 5-day program with three distinct phases consisting of preconditioning stage, conditioning and finally, the post-conditioning stage.[8] Preconditioning was applied on the start day of the conditioning. Each animal was placed individually into the box for ten minutes, with full access to both sides of the box. In conditioning stage, rats received morphine and saline, twice daily. On the first day of conditioning sessions (the 2nd day of CPP), the animals received morphine at 9 AM and remained in the box for 45 minutes. Six hours later, the rats received saline subcutaneously in another side of the compartment for 45 min. On day 2 (the 3rd day of CPP), the time order of receiving morphine and saline was reversed. On day 3 (the 4th day of CPP), the animals received morphine and saline as the first day. Immediately after each morphine or saline injection, the animals were placed in their relative compartments for 40 min, while they could not move to the other side of the box. Finally, post-conditioning phase started on the fifth day. The barrier between the two sides of the box was removed, and the animal placed in the middle of the box. Then, the time which each animal spend on each side of the box during 10 minutes was measured. Also, morphine or saline was not injected in animals during the test. CPP timeline for dose-response determination is shown in figure 2.

Analyzes were performed using the GraphPad Prism software (version 5, San Diego, CA, USA, 1994). Data comparison was performed using Tukey's range test and Student's t-test. Data were shown as means ± standard error of the mean (SEM).

Results

Determination of morphine dose response: Various quantities of morphine (0.5, 1, 2.5, 5, 7.5, 10 mg/kg of animal weight) were injected to the rats in the conditioning stage of CPP (Figure 3).

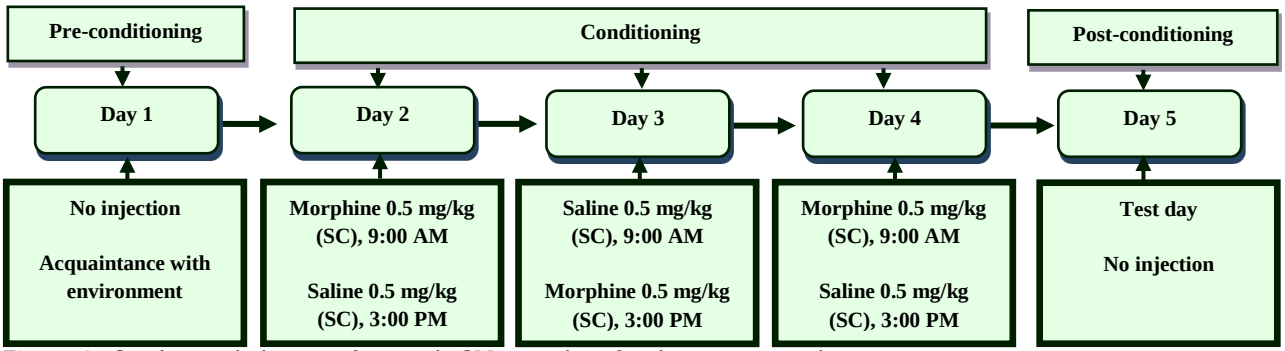

Figure 2. Conditioned place preferential (CPP) timeline for dose-response determination
In the plan, only one dose of morphine is shown (0.5 mg/kg, SC), the rest of dosages (1, 2.5, 5, 7.5, 10 mg/kg) were likewise examined
SC: Subcutaneous

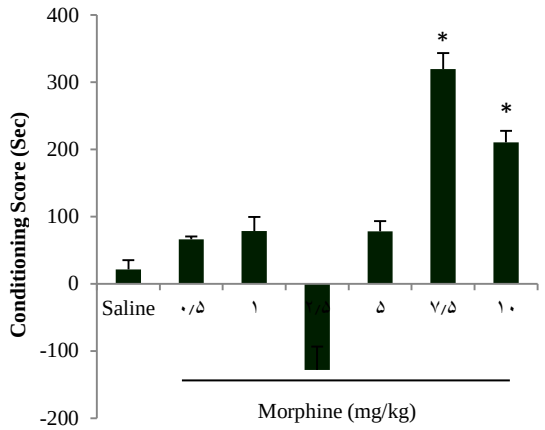

Figure 3. Morphine dose-response in conditioned place preferential (CPP) paradigm (n = 7)
Data are shown as mean ± standard error of the mean (SEM), *P < 0.001 for saline vs. 7.5 and saline vs. 10

Animals in doses 7.5 and 10 spent more time on the side of the box which was paired with morphine. To save morphine consumption, 7.5 mg/kg dosage of morphine, as an effective dose, and 2.5 mg/kg, as ineffective dose, were used in further stages.

Morphine effect on place conditioning in sensitized animals: Figure 4 shows the place conditioning effects on sensitization. The experimental group subcutaneously received an effective dose of morphine on the first three days of sensitization. Five days later, the CPP was done by morphine dosage 2.5 mg/kg. In the control group, the injection of saline in the first three days did not produce any sensitization.

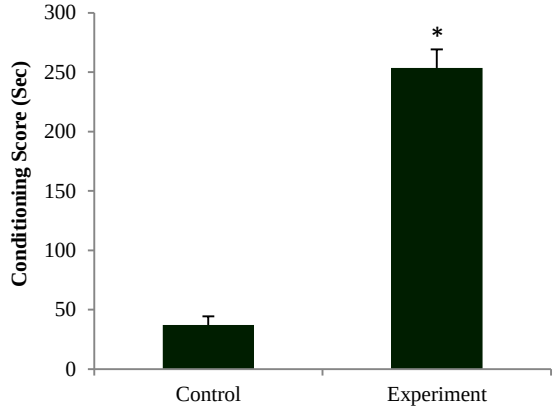

Figure 4. Comparison of conditioned place preferential (CPP) in sensitized and control groups (n = 7)
Data are shown as mean ± standard error of the mean (SEM), *P < 0.001

The effects of stimulation and inhibition of CeA $GABA_B$ receptors on the acquisition of morphine in morphine sensitive rats: To specify the effects of $GABA_B$ stimulation (by baclofen) or inhibition (via CGP35348) on the acquisition of morphine in CPP model of sensitive rats, certain doses of stimulator and inhibitor inserted into the core of the amygdala, ten minutes before each morphine taking in the first, second and third days of sensitization. The saline groups received saline instead of morphine during these 3 days. Five days later, the CPP was induced by 2.5 mg/kg dosage of morphine. Doses of 1.5, 6 and 12 µg/rat of baclofen had not significant effect on the sensitivity of animals to morphine at the acquisition stage. Injection of the inhibitor into the CeA (1.5, 6 and 12 µg/rat) causes a significant reduction in the acquisition level of morphine sensitivity in CPP model (Figures 5 and 6). Sensitization timeline is shown in figure 7.

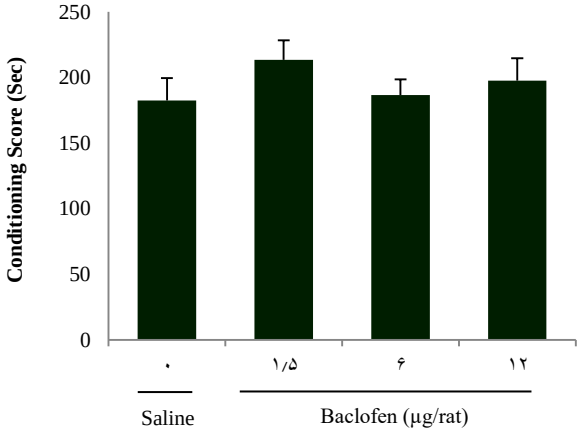

Figure 5. Effects of the intra-CeA administration of baclofen on the acquisition of conditioned place preferential (CPP) in morphine-induced sensitization (n = 7)
Data are shown as mean ± standard error of the mean (SEM)

Discussion

In confirmation of previous work on the positive effects of morphine reward in male[17] and female genders[8] in CPP model, taking morphine enhanced the time passed on the part of the device that was coupled with morphine, while saline injection did not have such an effect. Moreover, in our box, the animals did not show preference on either side of the box, which confirms un-biased CPP method.

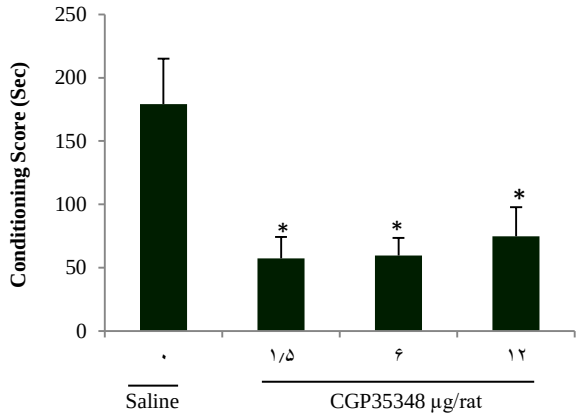

Figure 6. Effects of the intra-CeA administration of CGP35348 on the acquisition of conditioned place preferential (CPP) in morphine-induced sensitization (n = 7)

Data are shown as mean ± standard error of the mean (SEM), *P < 0.05 for saline vs. other groups

Sensitivity leads to multiple adaptive neuronal responses. Some of these adaptations include permanent changes in synaptic structures and altered gene expression.[18] Multiple neuromodulator and neurotransmitter including GABA[19] and dopamine have also been proposed to be involved in sensitization to morphine. Sensitivity by activating the GABAergic system can activate G-proteins which are used in the brain reward system. Sensitivity to morphine is due to pairing of G-protein and GABA receptors. This pairing leads to down-regulation of $GABA_B$ receptors and reduction of their inhibitory effects. Thus, sensitization with more craving for morphine increases the time spent in the part of the box that is coupled with morphine.[19]

The results of our study showed that sensitivity of females is more than the control group. Consistently, other reports also emphasized that females become addicted to morphine with lower dose, so their sensitivity is higher.[20]

Baclofen injection into the CeA had no significant effect on acquisition of sensitivity to morphine. Moreover, stimulating CeA $GABA_B$ receptors could not have any effect on morphine-induced sensitivity. A possible explanation is that $GABA_B$ receptor-dependent mechanisms in CeA might be in their maximum activity, or inversely, might not work at all during the sensitivity to morphine. These hypotheses can be further investigated by checking CGP35348 effects, showing that acquisition of sensitivity to morphine was significantly reduced in the presence of this $GABA_B$ antagonist. So, it implies that a maximum activity of $GABA_B$ receptors in CeA occurs at the time of sensitivity to morphine, and this activity is not expandable using baclofen. This controversial issue can be explained in such a way that there may be sub-types of the $GABA_B$ receptors in this area, which are important in the acquisition of morphine sensitivity in the sensitized female rats. The proposed $GABA_B$ receptors sub-types are $GABA_BR1a$, $GABA_BR1b$ and $GABA_BR2$. Advanced cell researches have shown that $GABA_BR1a$ sub-type is mostly located in pre-synaptic terminals, while $GABA_BR1b$ sub-type position is usually postsynaptically, and $GABA_BR2$ sub-type is mainly present at both post and pre-synaptic terminals. Pre-synaptic $GABA_B$ receptors activity result in repression of Ca^{2+} influx by inhibiting the cell membrane Ca^{2+} channels and decrease neurotransmitter release, while the activity of post-synaptic $GABA_B$ receptors leads to cells hyperpolarization by opening k^+ channels.

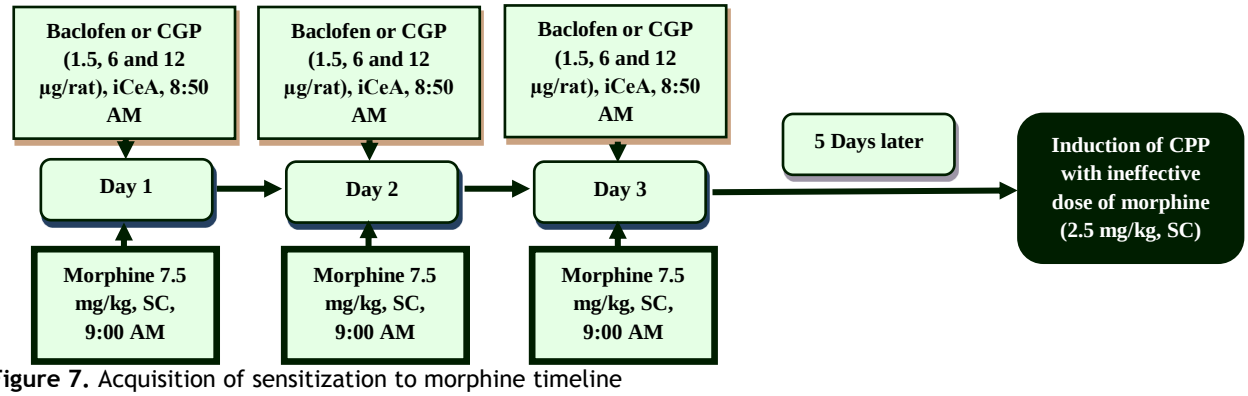

Figure 7. Acquisition of sensitization to morphine timeline

iCeA: Intra-CeA; SC: Subcutaneous; CPP: Conditioned place preferential

Some reports suggest that inhibition of $GABA_B$ receptors in the pre-synaptic terminals results in the release of more GABA transmitter, by which increases its inhibitory effects in the reward pathway.[21] Based on our results, it seems that CGP35348 might act on the pre-synaptic receptors and could cause a decrease in dopamine neurons activity and thereby, the reward pathway is inhibited.

Also, sex differences and hormonal fluctuations during the sexual cycle in female rats can influence the results.[22,23] Sex differences in morphine sensitization may be due to opioid receptors pharmacology,[24] density, binding and localization. Furthermore, diversities in the anatomy and physiology of neurons are also involved in the response to the drug.[23]

Finally, in morphine-sensitized rats the effects of GABA receptors agonist and antagonist in the target nucleus were not dose-dependent in CPP model, suggesting that all doses used in this study were saturating the receptors in the CeA.

Conclusion

The data of the present study implies that $GABA_B$ receptors within CeA may influence the process of behavioral sensitization, which results in the inhibition of morphine sensitization. Further studies with multidisciplinary approaches are necessary to identify the pre and post-synaptic mechanisms of the $GABA_B$ receptors.

Conflict of Interests

The Authors have no conflict of interest.

Acknowledgements

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References

1. Volkow ND. America's addiction to opioids: Heroin and prescription drug abuse. Proceedings of the 103th Congress of the United States of America, Senate Caucus on International Narcotics Control; 2014 Jun 25; Washington, DC, USA.
2. Shabani M, Divsalar K, Janahmadi M. Destructive effects of prenatal WIN 55212-2 exposure on central nervous system of neonatal rats. Addict Health 2012; 4(1-2): 9-19.
3. Nakhaee MR, Sheibani V, Ghahraman TK, Marefati H, Bahreinifar S, Nakhaee N. Does exercise deprivation increase the tendency towards morphine dependence in rats? Addict Health 2010; 2(3-4): 74-80.
4. Listos J, Baranowska-Bosiacka I, Wasik A, Talarek S, Tarnowski M, Listos P, et al. The adenosinergic system is involved in sensitization to morphine withdrawal signs in rats-neurochemical and molecular basis in dopaminergic system. Psychopharmacology (Berl) 2016; 233(12): 2383-97.
5. Pierce RC, Kalivas PW. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res Brain Res Rev 1997; 25(2): 192-216.
6. Torkzadeh-Mahani S, Nasri S, Esmaeili-Mahani S. Ginger (zingiber officinale roscoe) prevents morphine-induced addictive behaviors in conditioned place preference test in rats. Addict Health 2014; 6(1-2): 65-72.
7. Rafieirad M, Sahraei H, Haeri Rouhani SA, Sepehri H, Alavian Dehaghani SF, Ghoshouni H, et al. The modulatory role of gaba-b receptors of the shell part of nucleus accumbens in the acquisition and expression of morphine-induced conditioned place preference in morphine-sensitized rats. Physiology and Pharmacology 2007; 11(3): 182-91.
8. Sahraei H, Etemadi L, Rostami P, Pourmotabbed A, Zarrindast MR, Shams J, et al. GABAB receptors within the ventral tegmental area are involved in the expression and acquisition of morphine-induced place preference in morphine-sensitized rats. Pharmacol Biochem Behav 2009; 91(3): 409-16.
9. Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Brain Res Rev 1991; 16(3): 223-44.
10. Bijani S, Sadeghi-Gharachehdaghi S, Zardooz H, Ghoshooni H, Eidi A, Shams J, et al . Influence of nitric oxide in the central amygdala on the acquisition and expression of morphine-induced place preference in morphine sensitized rats. Basic Clin Neurosci 2011; 2(4): 36-46.
11. Karimi S, Radahmadi M, Fazilati M, Azizi-Malekabadi H, Alaei H. Roles of the nucleus accumbens (shell) in the acquisition and expression of morphine-induced conditioned behavior in freely moving rats. Int J Prev Med 2014; 5(3): 262-8.
12. De Vries TJ, Shippenberg TS. Neural systems underlying opiate addiction. J Neurosci 2002; 22(9): 3321-5.
13. Fu Z, Yang H, Xiao Y, Zhao G, Huang H. The gamma-aminobutyric acid type B (GABAB)

receptor agonist baclofen inhibits morphine sensitization by decreasing the dopamine level in rat nucleus accumbens. Behav Brain Funct 2012; 8: 20.
14. Arout CA, Caldwell M, Rossi G, Kest B. Spinal and supraspinal N-methyl-D-aspartate and melanocortin-1 receptors contribute to a qualitative sex difference in morphine-induced hyperalgesia. Physiol Behav 2015; 147: 364-72.
15. Karami M, Zarrindast MR, Sepehri H, Sahraei H. Role of nitric oxide in the rat hippocampal CA1 area on morphine-induced conditioned place preference. Eur J Pharmacol 2002; 449(1-2): 113-9.
16. Paxinos G, Franklin KBJ. The mouse brain in stereotaxic coordinates. 2nd ed. Houston, TX: Gulf Professional Publishing; 2004.
17. Zarrindast MR, Karami M, Sepehri H, Sahraei H. Influence of nitric oxide on morphine-induced conditioned place preference in the rat central amygdala. Eur J Pharmacol 2002; 453(1): 81-9.
18. Koob GF, Bloom FE. Cellular and molecular mechanisms of drug dependence. Science 1988; 242(4879): 715-23.
19. Narita M, Shibasaki M, Mizuo K, Suzuki T. Changes in G-protein activity mediated through the stimulation of dopamine and GABA(B) receptors in the mesolimbic dopaminergic system of morphine-sensitized mice. Addict Biol 2003; 8(3): 319-25.
20. Karami M, Zarrindast MR. Place aversion by morphine in offspring born of female morphine administered Wistar rats. Iran J Pharm Res 2011; 10(3): 577-84.
21. Bettler B, Kaupmann K, Mosbacher J, Gassmann M. Molecular structure and physiological functions of GABA(B) receptors. Physiol Rev 2004; 84(3): 835-67.
22. Kianpoor M, Bakhshani N. Trauma, dissociation, and high-risk behaviors. Int J High Risk Behav Addict 2012; 1(1): 9-13.
23. Becker JB, Hu M. Sex differences in drug abuse. Front Neuroendocrinol 2008; 29(1): 36-47.
24. Craft RM, Kalivas PW, Stratmann JA. Sex differences in discriminative stimulus effects of morphine in the rat. Behav Pharmacol 1996; 7(8): 764-78.

اثرات گیرنده‌های گابا نوع B هسته مرکزی آمیگدال بر روی کسب ترجیح مکان شرطی شده ناشی از مورفین در موش‌های ماده حساس شده

دکتر فیروزه علویان[1]، دکتر سعیده قیاسوند[2]، دکتر هدایت صحرایی[3]، دکتر مریم رفیعی راد[4]

مقاله پژوهشی

چکیده

مقدمه: آمیگدال یکی از مهم‌ترین مراکز عصبی درگیر در پاداش دارویی می‌باشد و هسته مرکزی آن ممکن است در پاداش دارویی مورفین درگیر شود. سیستم Gamma-aminobutyric acid-ergic (GABAergic) واقع در هسته مرکزی آمیگدال، نقش مهمی در تعدیل اثرات پاداشی مورفین دارد. هدف از انجام مطالعه حاضر، بررسی اثرات تحریک یا مهار گیرنده‌های گابا نوع B بر کسب حساسیت به مورفین با استفاده از روش ترجیح مکان شرطی شده در موش‌های رت ماده نژاد ویستار بود.

روش‌ها: رت‌های ماده نژاد ویستار تهیه شده از دانشگاه شهید بهشتی به ۱۷ گروه تقسیم شدند. تعداد حیوانات در هر گروه ۷ عدد در نظر گرفته شد (۷ گروه برای تعیین دوز ریسپانس، دو گروه شاهد و حساسیت و ۸ گروه برای دوزهای مختلف آگونیست و آنتاگونیست در مرحله کسب). همچنین، دوزهای ۰/۵، ۱، ۲/۵، ۵، ۷/۵ و ۱۰ میلی‌گرم بر کیلوگرم مورفین به صورت زیرجلدی و به منظور شناخت دوزهای مؤثر و بی‌اثر مورفین استفاده گردید. ۵ روز پس از جراحی، القای حساسیت صورت گرفت. باکلوفن (۱/۵، ۶ و ۱۲ میکروگرم برای هر رت) یا CGP35348 (با همان دوزهای آگونیست) ۱۰ دقیقه قبل از تزریق مورفین به داخل هسته مرکزی آمیگدال تزریق شد و پس از حساسیت، مراحل پیش‌شرطی سازی طی گردید.

یافته‌ها: تزریق باکلوفن تأثیر معنی‌داری بر کسب حساسیت به مورفین نداشت. در مقابل، تزریق CGP35348 منجر به کاهش کسب حساسیت به مورفین شد.

نتیجه‌گیری: انتقال دهنده گابا می‌تواند از طریق گیرنده‌های خاص، در کاهش میل به مورفین مؤثر باشد. بنابراین، این جایگاه‌ها می‌تواند هدف درمانی مهمی در مقابله با اثرات سوء مواد مخدر باشد.

واژگان کلیدی: مورفین، باکلوفن، رت‌ها، آمیگدال

ارجاع: علویان فیروزه، قیاسوند سعیده، صحرایی هدایت، رفیعی راد مریم. **اثرات گیرنده‌های گابا نوع B هسته مرکزی آمیگدال بر روی کسب ترجیح مکان شرطی شده ناشی از مورفین در موش‌های ماده حساس شده.** مجله اعتیاد و سلامت ۱۳۹۶؛ ۹ (۲): ۱۱۷-۱۱۰.

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