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Investigation of melatonin receptors gene expression levels in the hippocampus and hypothalamus in rats with an experimental morphine dependence model

Yasin Ali Cimen[a,b], Faik Ozdengul[a,c], Canan Eroglu Gunes[c,d], Ercan Kurar[c,d], Zulfikare Isik Solak Gormus[a,c], Kaniye Zeynep Caliskan Sak[a,e], Selim Kutlu[a,c,*]

[a] *Necmettin Erbakan University, Faculty of Meram Medicine, Department of Physiology, Konya, Türkiye*
[b] *Bezmialem Vakif University, Faculty of Medicine, Department of Physiology, Istanbul, Türkiye*
[c] *Necmettin Erbakan University Neuroscience Research and Application Center, Konya, Türkiye*
[d] *Necmettin Erbakan University, Faculty of Medicine, Department of Medical Biology, Konya, Türkiye*
[e] *KTO Karatay University, Faculty of Medicine, Department of Physiology, Konya, Türkiye*

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Abstract

Aim: Morphine is one of the important opioids used in chronic and acute pain management. However, it has many side effects, including the development of addiction, which seriously limits its use. Melatonin shows its physiological effects through melatonin receptor 1 (MT1), melatonin receptor 2 (MT2) and heterodimer receptors (MT1/MT2). No study has examined the presence of MT1/MT2 mRNA in the hypothalamus and hippocampus and its relationship with the addiction process. The aim of this study was to investigate the gene expression levels of MT1, MT2 and MT1/MT2 in rat hippocampus and hypothalamus during morphine addiction and morphine withdrawal.

Materials and Methods: A total of 36 male Wistar rats were divided into 3 groups (n=12). Control (C) group received saline subcutaneously for 6 days. Morphine (M) and morphine+naloxone (M+N) groups received 10 mg/kg/day morphine subcutaneously for 6 days. On the seventh day, saline was injected intraperitoneally into C and M groups and 1 mg/kg naloxone was injected intraperitoneally into M+N group. 30 minutes later, hippocampus and hypothalamus tissues of rats were dissected. Melatonin receptor genes expression level were analysed by quantitative qPCR.

Results: Both MT1 and MT1/MT2 gene expression levels in the hypothalamus were higher in the M+N group than in the C group ($p<0.05$). There was no difference between the expression levels of MT2 receptors in the hypothalamus ($p>0.05$). There was no difference in MT1, MT2 and MT1/MT2 gene expression in the hippocampus ($p>0.05$).

Conclusion: This is the first study to show the presence of MT1/MT2 in the hypothalamus and hippocampus, and it is possible that MT1 and MT1/MT2 receptors, especially in the hypothalamus, play a role in the addiction process.

Introduction

Usually, morphine is an opioid used to treat short or long term pain [1]. The most important side effects of morphine treatment are the development of tolerance and hyperalgesia. These side effects occur in long-term morphine intake and limit its use [2]. In addition to these adverse effects, complications such as respiratory depression, constipation and addiction also restrict the long-term use of morphine [3]. In addition, chronic morphine consumption has often been associated with changes in brain biochemistry and hormone status and disruption of the sleep/wake cycle [4].

Naloxone is an opioid receptor antagonist. Naloxone is used to observe rapid morphine withdrawal symptoms in morphine withdrawal studies. Thanks to this feature, it is frequently used in the diagnosis, treatment and morphine addiction model studies of opioid addiction and narcotic overdose poisoning [5].

Melatonin is a serotonin-originated neurohormone and is secreted almost exclusively from the pineal gland [6]. It is particularly popular for its role in modulating the circadian rhythm [7]. In addition to this effect, it functions in mood, sleep, reproduction, retinal functions, tumor growth, regulation of behavior, and neuroprotection [8]. It is known that melatonin exerts its physiological effects in mammals through G protein-coupled melatonin receptor 1

*Corresponding author:
Email address: skutlu@erbakan.edu.tr (Selim Kutlu)

(MT1), melatonin receptor 2 (MT2) and later melatonin heterodimer receptors (MT1/MT2) [9, 10]. MT1 mRNA is expressed in the hippocampus [11], hypothalamus, cerebellum, frontal cortex, nucleus accumbens, and amygdala [12]. MT2 mRNA is found in the hippocampus, hypothalamus, reticular thalamic nucleus, substantia nigra, pars reculat, and ventrolateral periaductal gray [13]. qPCR and immunohistochemical studies in transgenic animal models revealed the presence of MT1 and MT2 mRNA and protein expressions in granular and pyramidal neurons of the hippocampus [14]. The presence of MT1/MT2, which have night time light sensitivity and are responsible for the increase due to melatonin, has been reported in the mouse retina [9]. Due to the paucity of studies and lack of clear data, the localization of MT1/MT2 heterodimer especially in the brain is a matter of debate [15].

The addiction relationship between melatonin and melatonin receptors (MR) has been investigated in different studies. There is a strong association between disruption of circadian rhythm and addiction development [16]. Melatonin enhances the anti-nociceptive effect of opioid drugs and may reduce morphine tolerance [1]. A study with cocaine administration suggested that melatoninergic activity may play a role in opioid addiction [17]. Systemic or intrathecal administration of melatonin has been found to have a dose-dependent analgesic effect and reduce morphine-mediated hyperalgesia [18]. Another study reported that melatonin can modulate the expression of genes responsible for morphine tolerance [19]. In a recent study, simultaneous use of melatonin with morphine was reported to offer a potential way to help reduce the occurrence of morphine tolerance and hyperalgesia [20]. Information on changes in gene expression levels of MR in the hypothalamus and hippocampus during morphine addiction and withdrawal is limited. In addition to the unknown presence of mRNA for the MT1/MT2 receptor in the hippocampus and hypothalamus, no studies have described its association with addiction and drug withdrawal. In this study, we aimed to examine how the gene expression status of MR, which is known to play a role in the addiction process, changes in rat hippocampus and hypothalamus during morphine dependence and morphine withdrawal.

Materials and Methods

Test animals

In this study, 36 nonsibling or unrelated adult male rats were selected and categorized into three groups: control (C), morphine (M) and morphine+naloxone (M+N), 12 male rats in each group. To the control group, 0.9% NaCl solution (SF) was administered subcutaneously for 6 days, and a single dose was injected intraperitoneally on the morning of the 7th day. Rats in groups M and M+N were given a subcutaneous injection of 10 mg/kg/day [21] morphine (obtained from Necmettin Erbakan University Hospital of Medical Faculty) for 6 days. Intraperitoneal SF was administered to the morphine group on the morning of the 7th day, and 1 mg/kg naloxone (Merck Company, N7758, Kenilworth, USA) to the M+N group on the morning of the 7th day [22]. Animals were kept at 22±1°C with a 12-hour light/dark cycle. Food and tap water were provided ad libitum. All animal care and all experimental protocols were approved by Necmettin Erbakan University Experimental Medicine Application and Research Center in accordance with institutional and international standards. Ethics committee approval was obtained from Necmettin Erbakan University Experimental Animals Ethics Committee (2020-039). Animal rights are protected under the 'Guide for the Care and Use of Laboratory Animals'.

Experimental protocol

Total RNA isolation

After behavioural analysis, brain tissues of rats from all groups were rapidly removed. Hippocampus and hypothalamus brain regions were isolated. They were immediately frozen in liquid nitrogen and stored at -80°C for gene expression analysis. Total RNA isolation was performed using the TRIzol method. The density and quality of total RNA samples were assayed spectrophotometrically and by agarose gel electrophoresis. To eliminate possible gDNA contamination, DNAse-I (Thermo Scientific; EN0521) digestion was performed according to the manufacturer's directions. cDNA was synthesized from total RNA samples using Bio-Rad iScriptTM cDNA Synthesis Kit (#170-8891, USA).

Primer design and Quantitative real time-PCR analysis

Primers for MT1, MT2, MT1/MT2 and the reference genes (PGK1, RPL13A and GAPDH) with were generated using the IDT PrimerQuest (https://eu.idtdna.com/site) program or taken from the previous studies. Quantitative expression analysis of target and reference genes was performed using a real time PCR device (Bio-Rad CFX Connect Real Time PCR System). Briefly, polymerase chain reaction was performed by 10 µl of 2X SyberGreen master mix, 5 pMol each primer and 2 µl cDNA in 20 µl ddH_2O total volume. The temperature profile of the reaction was set as +95 ° C 10 min denaturation than 40 cycles of 95 ° C 30 sec, 60 ° C 30 sec and 72 ° C 30 sec.

Statistical analysis

Taking previous studies as reference, the minimum sample size was calculated as n=12 per group when the difference between means was taken as 0.4 and standard deviation as 0.1 for 90% power at 95% confidence level [24]. For the analysis of gene expression data, Ct values of all genes were normalized to the Ct values of PGK1, RPL13A and GAPDH reference genes and ΔCt values were calculated. Gene expression differences groups were expressed as mean ± SE values and analysed by one-way factorial ANOVA with Tukey post-hoc test. *$p<0.05$ is considered significant.

Results

Melatonin receptors gene expression results

According to the results of qPCR analysis of hippocampus tissues, although MT1 and MT2 gene expression levels were higher in the M group, there was no difference between the groups ($p>0.05$, Figure 1A-B). MT1/MT2

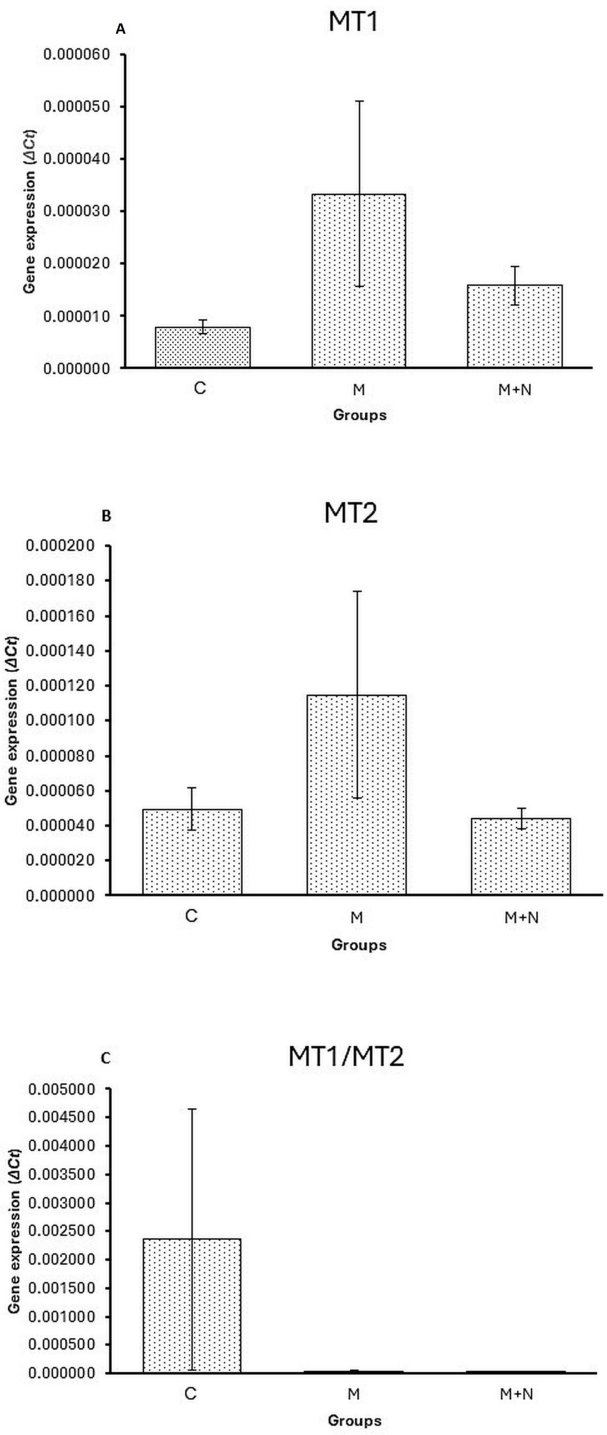

Figure 1. Gene expression levels of MT1 (A), MT2 (B) and MT1/MT2 (C) in the hippocampus Control (C), Morphine (M) and Morphine+naloxone (M+N) groups. (All data were expressed as mean ± SE values and analysed by one-way factorial ANOVA with Tukey post-hoc test. There is no significant difference between the groups ($p>0.05$).

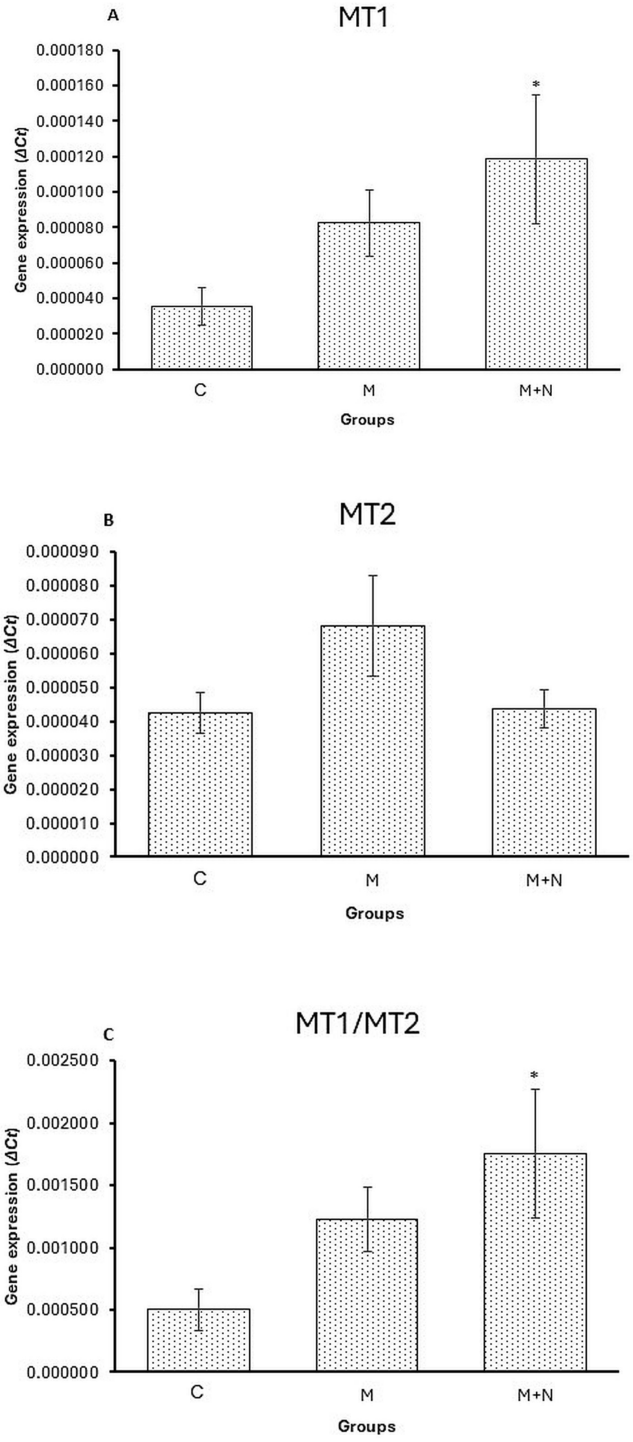

Figure 2. Gene expression levels of MT1 (A), MT2 (B) and MT1/MT2 (C) in the hypothalamus of Control (C), Morphine (M) and Morphine+naloxone (M+N) groups. graphically evaluated. (All data were expressed as mean ± SE values and analysed by one-way factorial ANOVA with Tukey post-hoc test. *$p<0.05$ is considered significant).

gene expression levels tended to increase in group C compared to other groups ($p>0.05$, Figure 1C). Both MT1 and MT1/MT2 gene expression levels in the hypothalamus were higher in the M+N group than in the C group ($p<0.05$, Figure 2A-C). MT2 receptor gene expression tended to increase in the M group ($p=0.07$, Figure 2B).

Discussion

Morphine is an opioid commonly used to treat severe pain. The first study in which melatonin was associated with pain dates back to 1969. It was reported that increased melatonin level in the dark suppressed the sensation of pain [25]. Studies on melatonin, MR, pain and opioids are still ongoing and remain popular. Different mechanisms have been proven for melatonin's suppression of pain. Melatonin has been found to have anti-nociceptive effects through hyperpolarization by increasing the flow of K^+ ions in the hippocampus and suprachiasmatic nucleus. It has been reported that Ca^{2+} channels in dorsal root ganglia, which play a role in neuropathic pain, are inhibited by melatonin [26]. The suppression of the pain-reducing effect of melatonin by naloxone proves that there are important mechanisms between melatonin and opioids [27]. In another study using a morphine addiction model, it was found that the inhibition of withdrawal behaviors observed in mice was prevented by melatonin by increasing endorphin content in the periaqueductal gray of the midbrain and reducing β-endorphin status in the arcuate nucleus [28]. The tolerance-reducing effect of melatonin in the morphine addiction model has been demonstrated by behavioral tests and it has been reported that the antioxidant role of melatonin may mediate this effect [19]. Melatonin enhances the anti-nociceptive effect of opioids and reduces the process of developing morphine tolerance [1]. These and similar studies led us to examine the interaction of morphine dependence, morphine withdrawal and MR. The hippocampus and hypothalamus are associated with addiction processes [29] and negative emotional states resulting from morphine withdrawal [30]. Therefore, we have shown the change of MR expression levels in these regions. Furthermore, the abundant presence of MT1 and MT2 in these regions [13] suggested that addiction-related MR expression changes in these regions should be investigated.

Melatonin functions predominantly by binding to MT1 and MT2. Therefore, it has become important to support the effect of melatonin on morphine addiction with MT1 and MT2 specific studies. Fan et al. observed a dramatic decrease in serum melatonin and MT1 mRNA after chronic morphine injection in rats, while MT2 gene expression in the spinal dorsal horn was unchanged [34]. In different studies, melatonin injection has been shown to suppress hyperalgesia via spinal MT2 receptors [35]. Functionality of the MT1 receptor rather than MT2 in the development of cocaine-mediated locomotor sensitization in rodents has been reported [36]. In an opioid and melatonin study with mice, it was reported that MT1 and MT2 mRNA levels in the hippocampus did not change in response to cocaine administration, while MT1 mRNA level in the striatum decreased and MT2 mRNA amount did not change. Interestingly, the effects of such substances on MT1/MT2 mRNA have been reported to be brain region specific [37]. In our qPCR results performed in hippocampus samples, although MT1 and MT2 gene expressions tended to increase in M groups, no difference was found in terms of MT1, MT2 and MT1/MT2 gene expressions (Figure 1A-B). MT1/MT2 receptor expression in the hippocampus tended to increase in M and M+N groups compared to the control (Figure 1C). The fact that the expression levels of all 3 receptor types tended to decrease in the M+N group compared to the other groups supports the information mentioned by Imbesi et al. We believe that this result may contribute to future studies of melatonin and addiction targeting the hippocampus.

Melatonin has been reported to prevent morphine dependence by increasing β-endorphin expression in the arcuate nucleus [38]. In the hypothalamus, we found high expression levels of MT1 and MT1/MT2 in the M+N group (Figure 2A-C). This result is evidence that MT1 and MT1/MT2 in particular contribute to the regulation of melatonergic activity compared to MT2 in the hypothalamus during morphine withdrawal. This result also suggests that these receptors in the hypothalamus play a more dominant role than the hippocampus in addiction and withdrawal processes. The proof that MT1/MT2 is present in these regions and plays a role in addiction processes in this study may lead to further studies.

Conclusion

In the light of this information, it can be said that MRs in the hippocampus and hypothalamus contribute to the effects of melatonin on morphine addiction. Considering our study, it can be concluded that gene expression levels of MRs involved in the process of morphine addiction may differ according to brain regions and may cause some neuroendocrinological effects. In addition to being the first study to demonstrate the presence of MT1/MT2 in the hypothalamus and hippocampus, the relationship between these receptors and addiction was also shown. The results may emphasis the importance of MRs in understanding the epigenetic etiology and pathogenesis of morphine addiction.

Ethical approval

Ethics committee approval was obtained from Necmettin Erbakan University Experimental Animals Ethics Committee (2020-039).

References

1. Song L, Wu C, Zuo Y. Melatonin prevents morphine-induced hyperalgesia and tolerance in rats: role of protein kinase C and N-methyl-D-aspartate receptors. BMC Anesthesiology. 2015;15:1-8.
2. Volkow ND, McLellan AT. Opioid abuse in chronic pain—misconceptions and mitigation strategies. NJM. 2016;374(13):1253-63.
3. Günther T, Dasgupta P, Mann A, et al. Targeting multiple opioid receptors–improved analgesics with reduced side effects? Br. J. Pharmacol. 2018;175(14):2857-68.
4. Wang Q, Yue XF, Qu WM, et al. Morphine inhibits sleep-promoting neurons in the ventrolateral preoptic area via mu receptors and induces wakefulness in rats. Neuropsychopharmacology. 2013;38(5):791-801.
5. Tsai RY, Cheng YC, Wong CS. Naloxone inhibits morphine-induced chemotaxis via prevention of heat shock protein 90 cleavage in microglia. J Formos Med Assoc. 2015;114(5):446-55.
6. Pang CS, Tsang SF, Yang JC. Effects of melatonin, morphine and diazepam on formalin-induced nociception in mice. Life Sci. 2001;68(8):943-51.
7. Mehrzadi S, Hemati K, Reiter RJ. Mitochondrial dysfunction in age-related macular degeneration: melatonin as a potential treatment. Expert Opin Ther Targets. 2020;24(4):359-78.

8. Vengeliene V, Noori HR, Spanagel R. Activation of melatonin receptors reduces relapse-like alcohol consumption. Neuropsychopharmacology. 2015;40(13):2897-906.
9. Baba K, Benleulmi-Chaachoua A, Journé AS, et al. Heteromeric MT1/MT2 melatonin receptors modulate photoreceptor function. Sci Signal. 2013;6(296):ra89.
10. Dubocovich ML, Masana MI, Benloucif S. Molecular pharmacology and function of melatonin receptor subtypes. Melatonin after four decades: Springer; 2002. p. 181-90.
11. Mazzucchelli C, Pannacci M, Nonno R, et al. The melatonin receptor in the human brain: cloning experiments and distribution studies. Mol Brain Res. 1996;39(1):117-26.
12. Uz T, Arslan AD, Kurtuncu M, et al. The regional and cellular expression profile of the melatonin receptor MT1 in the central dopaminergic system. Brain Res Mol Brain Res. 2005;136(1-2):45-53.
13. Lacoste B, Angeloni D, Dominguez-Lopez S, et al. Anatomical and cellular localization of melatonin MT1 and MT2 receptors in the adult rat brain. J Pineal Res. 2015;58(4):397-417.
14. Ng KY, Leong MK, Liang H. Melatonin receptors: distribution in mammalian brain and their respective putative functions. Brain Struct Funct. 2017;222(7):2921-39.
15. Klosen P, Lapmanee S, Schuster C, et al. MT1 and MT2 melatonin receptors are expressed in nonoverlapping neuronal populations. Pineal Res. 2019;67(1):e12575.
16. Patterson F, Grandner MA, Malone SK, et al. Sleep as a target for optimized response to smoking cessation treatment. Nicotine Tob Res. 2019;21(2):139-48.
17. Takahashi TT, Vengeliene V, Spanagel R. Melatonin reduces motivation for cocaine self-administration and prevents relapse-like behavior in rats. Psychopharmacology (Berl). 2017;234(11):1741-8.
18. Zurowski D, Nowak L, Machowska A, et al. Exogenous melatonin abolishes mechanical allodynia but not thermal hyperalgesia in neuropathic pain. The role of the opioid system and benzodiazepine-gabaergic mechanism. J Physiol Pharmacol. 2012;63(6):641-7.
19. Cheng YC, Tsai RY, Sung YT, et al. Melatonin regulation of transcription in the reversal of morphine tolerance: Microarray analysis of differential gene expression. Int J Mol Med. 2019;43(2):791-806.
20. Su L-Y, Liu Q, Jiao L, Yao Y-G. Molecular mechanism of neuroprotective effect of melatonin on morphine addiction and analgesic tolerance: an update. Mol Neurobiol. 2021;58(9):4628-38.
21. You Z, Ding W, Doheny JT, et al. Methylphenidate and morphine combination therapy in a rat model of chronic pain. Anesth. & Analg. 2020;130(2):518-24.
22. Raghav R, Jain R, Dhawan A, et al. Chronic co-administration of nalbuphine attenuates the development of opioid dependence. Pharmacol Biochem Behav. 2018;175:130-8.
23. Gellert VF, Holtzman SG. Development and maintenance of morphine tolerance and dependence in the rat by scheduled access to morphine drinking solutions. JPET. 1978;205(3):536-46.
24. Bongianni F, Carla V, Moroni F. Calcium channel inhibitors suppress the morphine-withdrawal syndrome in rats. Br. Pharmacol. 1986;88(3):561-7.
25. Morris RW, Lutsch EF. Daily susceptibility rhythm to morphine analgesia. Pharm Sci. 1969;58(3):374-6.
26. Ayar A, Martin DJ, Ozcan M. Melatonin inhibits high voltage activated calcium currents in cultured rat dorsal root ganglion neurones. Neurosci Lett. 2001;313(1-2):73-7.
27. Lissoni P, Esposti D, Esposti G, et al. A clinical study on the relationship between the pineal gland and the opioid system. J. Neural Transm. 1986;65:63-73.
28. Wei YM, Xu Y, Yu CX. Effect of melatonin on the content of β-endorphin in the hypothalamic arcuate nucleus and periaqueductal grey of midbrain in morphine withdrawal mice. Sheng li xue bao:[Acta Physiol Sin.]. 2007;59(6):765-9.
29. Noori HR, Spanagel R, Hansson AC. Neurocircuitry for modeling drug effects. Addict Biol. 2012;17(5):827-64.
30. Frenois F, Cador M, Caillé S, et al. Neural correlates of the motivational and somatic components of naloxone-precipitated morphine withdrawal. Eur J Neurosci. 2002;16(7):1377-89.
31. Li T, Hou Y, Cao W, et al. Naloxone-precipitated withdrawal enhances ERK phosphorylation in prefrontal association cortex and accumbens nucleus of morphine-dependent mice. Neurosci Lett. 2010;468(3):348-52.
32. Miyamoto Y, Takemori AE. Sites of action of naloxone in precipitating withdrawal jumping in morphine-dependent mice: investigations by the ED50 value and CNS content of naloxone. Drug and Alcohol Depend. 1993;32(2):163-7.
33. Tan-No K, Shimoda M, Sugawara M, et al. Cysteine protease inhibitors suppress the development of tolerance to morphine antinociception. Neuropeptides. 2008;42(3):239-44.
34. Fan Y, Liang X, Wang R. Role of endogenous melatoninergic system in development of hyperalgesia and tolerance induced by chronic morphine administration in rats. Brain Res. Bull. 2017;135:105-12.
35. Ambriz-Tututi M, Granados-Soto V. Oral and spinal melatonin reduces tactile allodynia in rats via activation of MT2 and opioid receptors. Pain. 2007;132(3):273-80.
36. Uz T, Javaid JI, Manev H. Circadian differences in behavioral sensitization to cocaine: putative role of arylalkylamine N-acetyltransferase. Life Sci. 2002;70(25):3069-75.
37. Imbesi M, Uz T, Yildiz S, et al. Drug- and region-specific effects of protracted antidepressant and cocaine treatment on the content of melatonin MT(1) and MT(2) receptor mRNA in the mouse brain. Int J Neuroprot Neuroregener. 2006;2(3):185-9.
38. Hassanipour M, Rajai N, Rahimi N, et al. Sumatriptan effects on morphine-induced antinociceptive tolerance and physical dependence: The role of nitric oxide. Eur J Pharmacol. 2018;835:52-60.