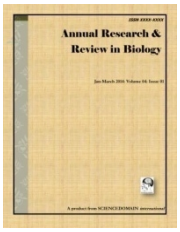

Annual Research & Review in Biology
4(24): 3932-3944, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluating the Effects of Estradiol Valerate and Raloxifene towards Morphine State-Dependent Learning in Mice

Mahdieh Anoush[1], Ali Jani[1] and Mohammad Reza Jafari[2*]

[1]Department of Pharmacology and Toxicology, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.
[2]Department of Physiology and Pharmacology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Author MA managed both the literature searches as well as the experiments, and prepared the manuscript. Author AJ performed the statistical analysis as and the experiments. Author MRJ designed the study, managed the analyses of the study and wrote the protocol. All authors read and approved the final manuscript.

Original Research Article

Received 25th March 2014
Accepted 10th June 2014
Published 17th July 2014

ABSTRACT

Aims: The aim of this study was to evaluate the possible effects of estradiol valerate and a specific estrogen receptor modulator, raloxifene on passive avoidance learning in male mice and to determine whether there is an interaction between estrogen receptor binding drugs and morphine.
Study Design: In this study, the state-dependent learning for morphine was evaluated at the first step, and then the effects of raloxifene and estradiol on this characteristic of morphine was evaluated. Besides the later two drugs' independent effect on passive avoidance learning was studied too.
Place and Duration of Study: All the experiments carried out in the pharmacology laboratory, School of Pharmacy, Zanjan University of Medical Sciences between April-July 2013.
Methodology: In this study, 120 Male NMRI mice were used according to the guidelines for animal ethics. State dependent learning (as one of the usual methods for assessment of passive avoidance learning) was performed using a step down shuttle box. During training,

**Corresponding author: Email: jafarimrj@yahoo.com, anoushm@zums.ac.ir;*

mice were shocked only after they step down on from the queue. Latencies to step down are recorded to assess passive avoidance short term memory. Prior training they received saline or morphine and before testing session the animals received saline or morphine plus estrogen valerate or raloxifene in relevant groups.
Results: The results illustrated that pre-training administration of morphine, induced amnesia which was retained by a similar pre-test dose (morphine state dependent learning). Pre-test estradiol valerate injection was able not only to retrieve morphine induced amnesia but also improved morphine state-dependent learning. On the other hand, raloxifene had no effect on memory retrieval itself, but decreased morphine state dependent learning.
Conclusion: The results of this study indicated that estrogen receptor modulators not only are able to manipulate the learning patterns, but also there might be an interaction between these drugs and morphine induced state dependent learning in mice.

Keywords: Estradiol; raloxifene; step-down; memory; morphine; mice.

1. INTRODUCTION

Learning can be described as the mechanism by which new information is acquired, while memory is the process of preserving the new information [1]. It is convenient to categorize memory as being explicit (conscious recall of information about people, places, and things), or implicit as the non-conscious recall of tasks such as motor skills [2].

State dependent learning has been known since 1930 and is described as a phenomenon in which the retrieval of short term memory is possible only if the subject is in the same physiological state [2-3]. Lots of drugs especially those with major role in the central nervous system (CNS), suggested to have state-dependent learning characteristic among which morphine is one of the most widely used [4]. Passive avoidance is a method developed for the study of learning and memory in mice based on the measurement of step-down latencies [5].

Morphine is an opioid with wide therapeutic usage pain relief [6]. Although, chronic use of it leads to physical and psychological dependences; evidence shows that morphine has a major site of action at midbrain, which makes it capable of affecting memory and learning patterns both in acute and chronic administration [7-10]. The majority of morphine-central actions such as state-dependent learning are mediated by mu-opioid receptors [11]. Opioid receptors are abundant in the hippocampus and are known to modulate the excitability of hippocampal neurons whereas mu-opioid receptor antagonists impair spatial learning and memory [12-13].

Binding of morphine with mu-opioid receptors at midbrain may lead to a dopamine release at the end of the neuronal projections in amygdala, which induces a rewarding effect of opioid [14-15]. It has been proved that several effects of acute and chronic exposure to morphine are expressed differently on the basis of gender such as anti-nociception [16], locomotion [17] and development of tolerance and dependence [18]. Moreover, according to the previous researches estrogen has been shown to influence learning and memory [19], while its efficacy varies with task study design [20], type of memory [21], and the rout or longevity of hormone administration [22].

It has been shown that estrogen plays a substantial role in the induction of acute tolerance to morphine induced analgesia [23]. Moreover, it has been reported that consolidation and retrieval of morphine-associated contextual memory can be disrupted by tamoxifen and this impairing effect might be prohibited by estradiol treatment [24]. On the other hand, spinal kappa- and mu-opioid receptor hetero-dimerization can be regulated via spinal synthesis of estrogen and concomitant signaling by membrane estrogen receptors as well as female-specific spinal morphine antinociception [25]. Although lots of researches have been carried out about estrogen and morphine interactions; there is no report suggesting the effects of estrogen towards morphine induced state-dependent learning.

As there is a gap of knowledge about the interaction between state dependent learning and SERMs and the lack of information on the raloxifene's effects on memory in general and state dependent learning in particular; the purpose of this study was to investigate the effects of various doses of estradiol valerate and raloxifene (a selective estrogen receptor modulator; SERM) for the first time on morphine state-dependent learning according to step-down passive avoidance task in mice.

2. MATERIALS AND METHODS

2.1 Animals

Male adult NMRI mice (bred in animal department, School of Pharmacy, with ISO17025 license) weighing 24.6–29.6 g were used in the present study. The animals were housed in a temperature/moisture controlled (22±3ºC/45-55% humidity) colony room. They were maintained in a 12-h light/dark cycle with food and water ad libitum, except during experimental procedures. All experiments were carried out between 10:00 a.m and 3:00 p.m. All subjects were acclimatized to the laboratory conditions for at least 72 hours prior to the initiation of any experiment. Each mouse was used once and each treatment group consisted of ten animals. All procedures were carried out according to national guidelines for animal care and use. The protocol was approved by the Committee of Ethics of the institute.

2.2 Drugs and Chemicals

Morphine sulphate was purchased from Temad (Iran). Estradiol valerate, raloxifene and ultra filtrated sesame oil (as a vehicle for estradiol) were purchased from Iran Hormone Company. Polyethylene glycol 300 (PEG300) was purchased from Merck Schuchardt OHG (Hohenbrunn, Germany).

Morphine sulfate was dissolved in normal saline (0.9%) and estradiol valerate emulsified in sesame oil and normal saline (0.9%). PEG300 was applied as a vehicle for raloxifene.

2.3 Passive Avoidance Apparatus

The passive avoidance apparatus consisted of a wooden box (30×30×40 cm height), the floor of which was consisted of 29 parallel stainless steel bars (0.3 cm in diameter, spaced 1 cm apart). A wooden platform (4×4×4 cm) was set in the center of the grid floor. Intermittent electric shocks (1 Hz, 0.5 sec, 50 V DC) were delivered to the grid floor by an insulated stimulator (Panlab LE12106, Spain). A single-trial step-down passive avoidance task was carried out applying this apparatus. Each mouse was gently placed on the wooden platform. When the mouse stepped-down from the platform and placed all its four paws on the grid

floor, the animal received electric shock for 15 sec. But in this weak voltage the mice only feel a transient general tremor and their long term reaction will be avoiding to step dawn the wooden queue in order to avoid experiencing this tremor. In order to set the retention test, each mouse was placed on the platform again at 24 h after training and the step-down latency was recorded with a stopwatch. An upper cut-off time of 300 sec was set for time recording.

2.4 Experiments

All treatments/drugs were injected intraperitoneally (I.P) and the dosage ranges were selected on the basis of references [26-27]. Although there was lack of information for raloxifene dosing in memory assessment models, the present dose range was applied according to three more relevant papers [28-30].

2.4.1 Morphine State-dependent learning (experiment 1)

This experiment examined morphine state dependent learning. Animals in control group received 10 ml/kg normal saline subcutaneously both in pre-training and pre-test administrations. Three other groups received 5mg/kg pre-training morphine followed by a pre-test administration of either saline or morphine (1.25, 2.5 and 5 mg/kg).

2.4.2 The effects of estradiol on morphine state-dependent learning (experiment 2)

On the training day, one group of animals received saline and other animals received morphine (5 mg/kg) 30 min before training. On the test day, animals received saline or estradiol (0.45, 0.9 and 1.8 mg/kg) in the presence or absence of morphine (5 mg/kg), 30 min before testing.

2.4.3 The effects of raloxifene on morphine state-dependent learning (experiment 3)

On the training day, one group of animals received saline; other animals received morphine (5 mg/kg) 30 min before training. On the test day, animals received saline or raloxifene (5, 10 and 20 mg/kg) 45 min before memory testing in the presence or absence of morphine (5 mg/kg) 30 min before testing.

2.5 Data Analysis

The step-down latencies were expressed as the median and interquartile range. The data were analyzed by using Kruskal–Wallis non-parametric one-way analysis of variance (ANOVA) followed by two-tailed Mann–Whitney U-test completed by a Holm's Bonferroni correction for the paired comparisons to evaluate significance of the results obtained. In all statistical evaluations $P<0.05$ was used as the criterion for statistical significance.

3. RESULTS

3.1 Morphine State-Dependent Learning

The results illustrated that pre-training administration of morphine (5 mg/kg) impaired the memory retrieval on the test day in comparison with saline-treated group (Kruskal-Wallis Non-Parametric ANOVA; $H(3) = 32.69$, $P < 0.001$) (Fig. 1A), while the memory retrieval

restored in groups which received different doses of morphine (1.25, 2.5 & 5 mg/kg) as a pre-test treatment (morphine state-dependent learning) (Kruskal-Wallis Non-Parametric ANOVA; H (3) = 35.11, $P < 0.001$, Mann Whitney U-test, $P < 0.001$ for different doses of morphine as test treatment) (Fig. 1B).

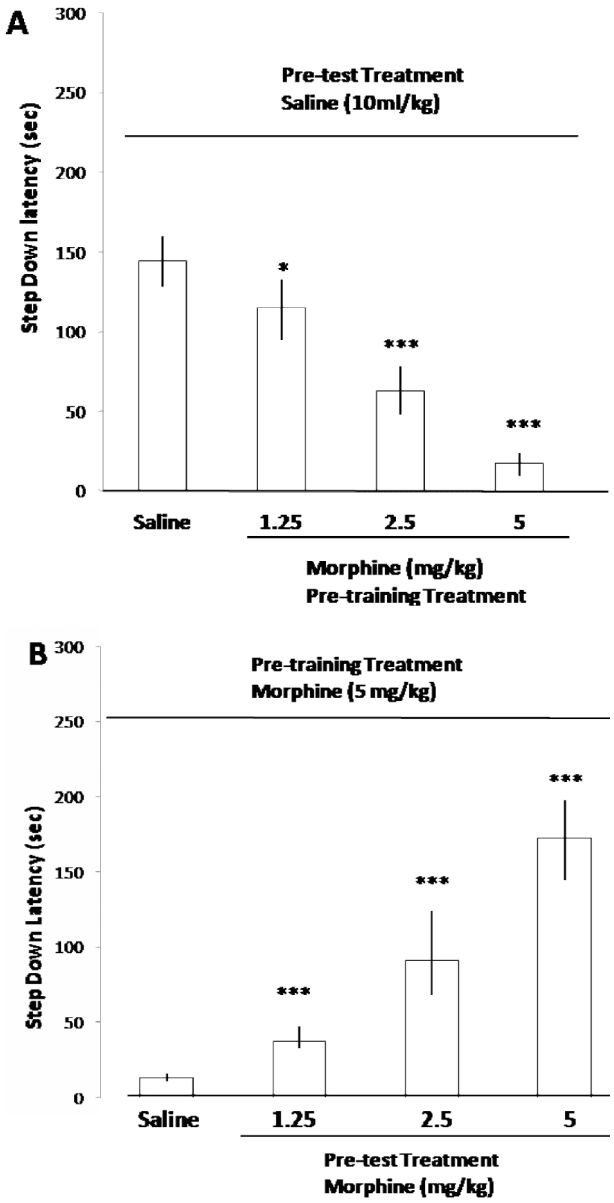

Fig. 1. The effect of (pre-training, different doses of morphine + pre-test saline) (1A) or (pre-training, 5 mg/kg morphine + pre-test different doses of morphine) (1B) on the step-down latencies compared to control groups

*Each value represents the median and quartile of 10 animals. * $P < 0.05$ and *** $P < 0.001$ compared to pre-training and pre-test saline in figure 1A. *** $P < 0.001$ compared to pre-training morphine (5 mg/kg) and pre-test saline in figure 1B*

3.2 The Effects of Estradiol on Learning

As shown in Fig. 2 (the left columns), pre-test administration of estradiol valerate (0.45, 0.9 and 1.8 mg/kg) enhanced memory retrieval which had been impaired by pre-training morphine (Kruskal-Wallis Non-Parametric ANOVA; H (3) = 15.68, *P* = 0.001). The best result was obtained with 1.8 mg/kg of estradiol valerate (Mann Whitney U-test, *P* = 0.143, 0.104 and 0.0006 for 0.45, 0.9 and 1.8 mg/kg of estradiol valerate respectively). Moreover, Fig. 2 (the right columns) illustrated that, pre-test co-administration of different doses of estradiol valerate (0.45, 0.9 and 1.8 mg/kg) with morphine (5 mg/kg), enhanced the memory retrieving effect of pre-test morphine (5 mg/kg), in comparison with vehicle + morphine-treated animals (Kruskal-Wallis Non-Parametric ANOVA; H (3) = 20.11, *P* < 0.001). Lower doses of estradiol valerate (0.45 and 0.9 mg/kg) had no significant effect on morphine state-dependent learning while the highest dose (1.8 mg/kg) significantly increased the retention time (Mann Whitney U-test, *P* = 0.684, 0.086 and 0.012 for 0.45, 0.9 and 1.8 mg/kg of estradiol valerate respectively).

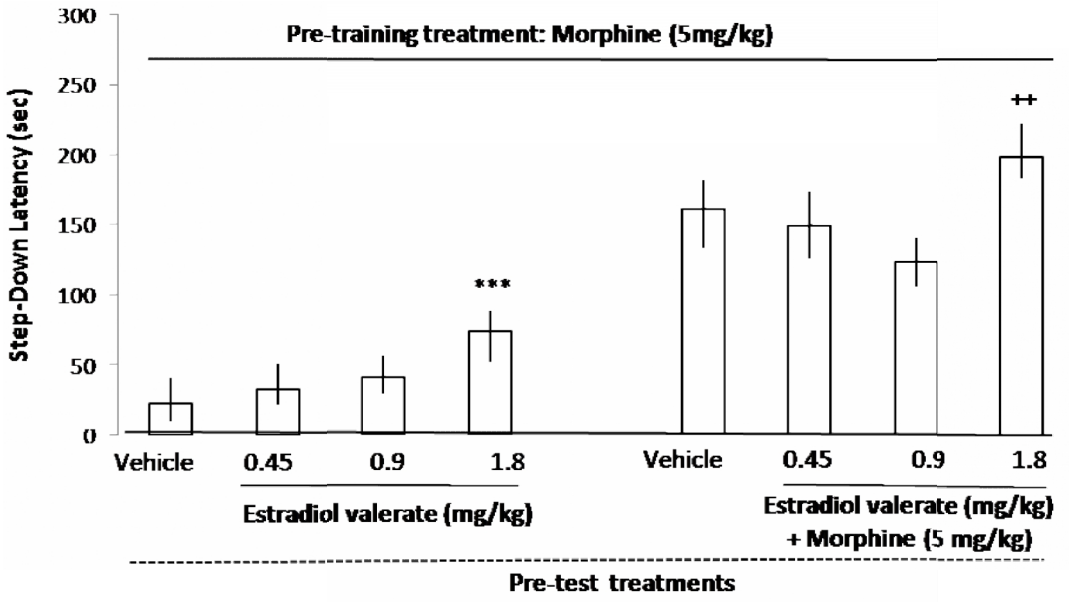

Fig. 2. The effect of pre-test administration of different doses of estradiol valerate or estradiol valerate + morphine after the administration of pre-training morphine

*Each value represents the median and quartile of 10 animals. *** P < 0.001 compared to pre-training morphine (5 mg/kg) and pre-test vehicle of estradiol valerate. ++ P < 0.01 compared to pre-training and pre-test morphine (5 mg/kg)*

3.3 The Effects of Raloxifene on Learning

According to Fig. 3 (the left columns), pre-test administration of raloxifene (5, 10 and 20 mg/kg, 45 min prior to test) did not retrieved memory which was impaired by pre-training 5 mg/kg of morphine (Kruskal-Wallis Non-Parametric ANOVA; H (3) = 3.6, *P*= 0.308). On the other hand, Fig. 3 (the right columns) illustrated that, pre-test co-administration of raloxifene (5, 10 and 20 mg/kg, 45 min prior to test) with morphine (5 mg/kg, 30 min prior to test), diminished the memory retrieval effect of pre-test morphine (state-dependent learning of

morphine), in comparison with vehicle-treated group (Kruskal-Wallis Non-Parametric ANOVA; H (3) = 10.7, $P < 0.014$). Lower doses of raloxifene (5 and 10 mg/kg) had no significant effect on memory retrieval by morphine while the highest dose (20 mg/kg) significantly decreased the retention time (Mann Whitney U-test, $P = 0.631$, 0.56 and 0.015 for 5, 10 and 20 mg/kg of raloxifene respectively).

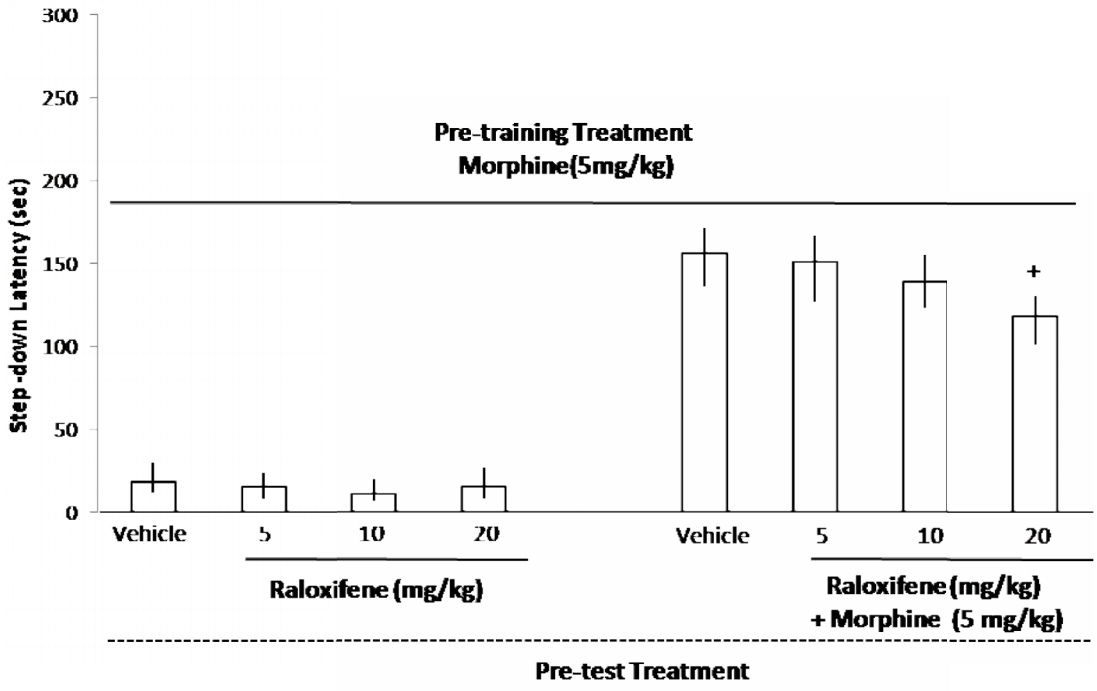

Fig. 3. The effect of pre-test administration of different doses of raloxifene or raloxifene + morphine after the administration of pre-training morphine

Each value represents the median and quartile of 10 animals. + P < 0.05 compared to pre-training and pre-test morphine (5 mg/kg)

4. DISCUSSION

The precise mechanisms of the interactions between systems related to memory are relatively unknown and currently under extensive investigation. In the present study, the effects of estrogen receptor modulators (estradiol valerate and raloxifene) on morphine induced amnesia and state-dependent learning were studied.

4.1 Morphine State-Dependent Learning

The results obtained from this research on morphine state-dependent learning illustrated that Pre-training administration of morphine (induced amnesia which was restored after a similar pre-test dose of the drug. These findings confirm the state dependent learning effect of morphine which was first introduced in 1990 [11] and later on, confirmed by other studies [31-35].

Findings on opioids role in memory, are controversial. While it has been reported that spatial memory [36] or synaptic plasticity [37] has been impaired by morphine infusion into medial septum. On the other hand, some findings proved that opioids can improve synaptic plasticity in hippocampus [38-39].

4.2 The Effects of Estradiol on Learning

The results of this study indicated that pre-test administration of estradiol valerate enhanced memory retrieval which was impaired by pre-training morphine administration. A growing body of evidence suggests that the most abundant gonadal steroid hormone (17ß-estradiol) in females may have an impact on memory and learning such as motor skills and spatial memory. The involved mechanisms are potentiating cerebellar plasticity in motor skills. Spatial memory enhancement selectively related to alpha estrogen receptors in the hippocampus [40-41]. Recent studies pointed out that estrogens potentiated spatial reference memory [20] as well as working memory [21]. Reports on learning and memory have been focused primarily on hippocampus, amygdale and cerebral cortex as basic sites in the brain where the plasticity occurs [42]. The hippocampus is an essential neural structure involved in the formation of certain types of memory [43].

Moreover, the present study showed that estradiol valerate improved morphine state-dependent learning (Fig. 2). A couple of studies have been carried out to examine other drugs or receptors' influence on morphine state dependent learning [7-8, 44-45], but there is no evidence till now, on estrogens' effects towards morphine state dependent learning. Meanwhile there are some reports on morphine and estrogen interactions. A research has mentioned that performance of adult male and female rats on tasks requiring learning and spatial memory, has been altered on the basis of sex differences by prenatal morphine exposure [46]. Morphine-induced conditioned place preference (CPP) in a dose-dependent manner increased by post-training or pre-testing administration of estradiol [24]. Morphine enhanced both the spatial memory performance in rats and Morris water maze test ranks in mice which were impaired by iAbeta packaged virus injection. This effect was carried out by induction of estradiol release in hippocampal neurons which suggested as a mechanism of morphine protection [47].

4.3 The Effects of Raloxifene on Learning

In this study, I.P injection of raloxifene 45 minutes prior to test did not show any effect on pre-training morphine induced amnesia. According to literature, there are contradictory reports regarding raloxifene effects on memory. it has been reported that raloxifene treatment did not impair cognition or affect mood in postmenopausal women [48]; meanwhile, brain activation patterns upon visual encoding in postmenopausal women were affected by raloxifene [49]. Besides, overall cognitive scores in postmenopausal women with osteoporosis have not been affected by three years of raloxifene treatment [50]. Raloxifene did not enhance spatial working memory in aged monkeys despite many years of estrogenic deprivation [51] and had no effect on dendritic branching during hippocampal development in vitro [52]. Another research mentioned that cognitive performance in ovariectomized rats had not been increased by raloxifene which was assessed by acquisition of a simple spatial memory task [29]. Eight weeks treatment with raloxifene had no effect on cognitive variables in postmenopausal women [53]. On the other hand it has been shown that raloxifene significantly increased neuronal outgrowth of hippocampal neurons within a narrow dose range but did not promote the outgrowth of basal forebrain or cortical neurons [54]. The drug

treatment in healthy elderly men enhanced brain activation in areas spanning a number of different cognitive domains which may related to effects on attention and working memory performance, executive functions, verbal skills, and episodic memory [55] in a way that may reflect increased arousal during initial encoding with downstream effects on brain function during retrieval of information [56]. Raloxifene-treated animals had significant positive effect on both memory deficit and the rate of recovery for the bilateral tactile removal test; also had a significant improvement in the acquisition of working memory [30]. Other studies reported that the drug not only had no negative influence on cognitive functioning in patients with breast cancer [57] but also significantly improved verbal memory in postmenopausal women when compared with placebo [58-59]. It has been shown that prefrontal cortex-related cognitive performance got better in the presence of raloxifene and it modulated prefrontal cortex morphology in ovariectomized rats [60]. Finally it is revealed that while lower dose of raloxifene improved verbal memory, higher doses produced a decrease in the risk of mild cognitive impairment and also lowered the risk of Alzheimer's disease in postmenopausal women [61].

In the present study, Pre-test raloxifene co-administration with morphine, did not affect morphine state-dependent learning, meanwhile higher doses of the drug diminished the above mentioned effect of morphine. There are little data regarding raloxifene (or SERM) and morphine interaction especially in the field of memory. It is revealed that tamoxifen was able to disrupt consolidation and retrieval of morphine-associated contextual memory and this impairing effect might be prohibited by estradiol treatment [24]. Raloxifene did not show any effect on morphine withdrawal induced hyperthermia in ovariectomized rats which was in contrast with 17 alpha-Ethinyl estradiol [62]. In the same model, the effect of Ethinyl estradiol in the morphine-dependent model of hot flush was inhibited by fulvestrant known as the full antagonist of estrogen receptors [63].

5. CONCLUSION

The results of this study indicated that estrogen receptor modulators not only are able to manipulate the learning patterns, but also, might have an interaction with morphine induced state dependent learning in mice.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Penfield W. Memory and perception. Res Publ Assoc Res Nerv Ment Dis. 1970;48:108-22.
2. Freyer F, Becker R, Dinse HR, Ritter P. State-dependent perceptual learning. J Neurosci. 2013;33(7):2900-7.

3. Siegel S. State dependent learning and morphine tolerance. Behav Neurosci. 1988;102(2):228-32.
4. Lowe G. State-dependent learning with social drugs. NIDA Res Monogr. 1991(116):267-76.
5. Nasehi M, Piri M, Jamali-Raeufy N, Zarrindast MR. Influence of intracerebral administration of NO agents in dorsal hippocampus (CA1) on cannabinoid state-dependent memory in the step-down passive avoidance test. Physiol Behav. 2010;100(4):297-304.
6. Narita M, Suzuki M, Niikura K, Nakamura A, Miyatake M, Yajima Y, et al. mu-Opioid receptor internalization-dependent and -independent mechanisms of the development of tolerance to mu-opioid receptor agonists: Comparison between etorphine and morphine. Neuroscience. 2006;138(2):609-19.
7. Ping X, Ma Y, Li Y, Qi C, Sun X, Lv X, et al. Essential role of protein kinase C in morphine-induced rewarding memory. Neuropharmacology. 2012;62(2):959-66.
8. Ardjmand A, Rezayof A, Zarrindast MR. Involvement of central amygdala NMDA receptor mechanism in morphine state-dependent memory retrieval. Neurosci Res. 2011;69(1):25-31.
9. Morphine and traumatic memory. Easing pain early on reduces risk of post-traumatic stress disorder. Harv Ment Health Lett. 2010;26(10):6.
10. Zarrindast MR, Noorbakhshnia M, Motamedi F, Haeri-Rohani A, Rezayof A. Effect of the GABAergic system on memory formation and state-dependent learning induced by morphine in rats. Pharmacology. 2006;76(2):93-100.
11. Nishimura M, Shiigi Y, Kaneto H. State dependent and/or direct memory retrieval by morphine in mice. Psychopharmacology (Berl). 1990;100(1):27-30.
12. Khajehpour L, Rezayof A, Zarrindast MR. Involvement of dorsal hippocampal nicotinic receptors in the effect of morphine on memory retrieval in passive avoidance task. Eur J Pharmacol. 2008;584(2-3):343-51.
13. Esmaeili MH, Kermani M, Parvishan A, Haghparast A. Role of D1/D2 dopamine receptors in the CA1 region of the rat hippocampus in the rewarding effects of morphine administered into the ventral tegmental area. Behav Brain Res. 2012;231(1):111-5.
14. Zarrindast MR, Rezayof A. Morphine state-dependent learning: sensitization and interactions with dopamine receptors. Eur J Pharmacol. 2004;497(2):197-204.
15. Rezayof A, Hosseini SS, Zarrindast MR. Effects of morphine on rat behaviour in the elevated plus maze: the role of central amygdala dopamine receptors. Behav Brain Res. 2009;202(2):171-8.
16. Juni A, Klein G, Kowalczyk B, Ragnauth A, Kest B. Sex differences in hyperalgesia during morphine infusion: effect of gonadectomy and estrogen treatment. Neuropharmacology. 2008;54(8):1264-70.
17. Craft RM, Clark JL, Hart SP, Pinckney MK. Sex differences in locomotor effects of morphine in the rat. Pharmacol Biochem Behav. 2006;85(4):850-8.
18. Hopkins E, Rossi G, Kest B. Sex differences in systemic morphine analgesic tolerance following intrathecal morphine injections. Brain Res. 2004;1014(1-2):244-6.
19. Hojo Y, Murakami G, Mukai H, Higo S, Hatanaka Y, Ogiue-Ikeda M, et al. Estrogen synthesis in the brain--role in synaptic plasticity and memory. Mol Cell Endocrinol. 2008;290(1-2):31-43.
20. Lipatova O, Toufexis DJ. Estrogen enhances the retention of spatial reference memory in the open field tower task, but disrupts the expression of spatial memory following a novel start position. Neurobiol Learn Mem. 2013;99:50-8.

21. Epperson CN, Amin Z, Ruparel K, Gur R, Loughead J. Interactive effects of estrogen and serotonin on brain activation during working memory and affective processing in menopausal women. Psychoneuroendocrinology. 2012;37(3):372-82.
22. Day M, Sung A, Logue S, Bowlby M, Arias R. Beta estrogen receptor knockout (BERKO) mice present attenuated hippocampal CA1 long-term potentiation and related memory deficits in contextual fear conditioning. Behav Brain Res. 2005;164(1):128-31.
23. Shekunova EV, Bespalov AY. Effects of memantine on estrogen-dependent acute tolerance to the morphine analgesia in female rats. Eur J Pharmacol. 2006;535(1-3):78-85.
24. Esmaeili B, Basseda Z, Gholizadeh S, Javadi Paydar M, Dehpour AR. Tamoxifen disrupts consolidation and retrieval of morphine-associated contextual memory in male mice: interaction with estradiol. Psychopharmacology (Berl). 2009;204(2):191-201.
25. Liu NJ, Chakrabarti S, Schnell S, Wessendorf M, Gintzler AR. Spinal synthesis of estrogen and concomitant signaling by membrane estrogen receptors regulate spinal kappa- and mu-opioid receptor heterodimerization and female-specific spinal morphine antinociception. J Neurosci. 2011;31(33):11836-45.
26. Stelly CE, Cronin J, Daniel JM, Schrader LA. Long-term oestradiol treatment enhances hippocampal synaptic plasticity that is dependent on muscarinic acetylcholine receptors in ovariectomised female rats. J Neuroendocrinol. 2012;24(6):887-96.
27. Craft RM, Ulibarri C, Leitl MD, Sumner JE. Dose- and time-dependent estradiol modulation of morphine antinociception in adult female rats. Eur J Pain. 2008;12(4):472-9.
28. Condi FL, Soares JM, Jr., Teodoro WR, Veloso AP, Parra ER, de Jesus Simoes M, et al. The effects of conjugated estrogen, raloxifene and soy extract on collagen in rat bones. Climacteric. 2012;15(5):441-8.
29. Gibbs RB, Gabor R, Cox T, Johnson DA. Effects of raloxifene and estradiol on hippocampal acetylcholine release and spatial learning in the rat. Psychoneuroendocrinology. 2004;29(6):741-8.
30. Kokiko ON, Murashov AK, Hoane MR. Administration of raloxifene reduces sensorimotor and working memory deficits following traumatic brain injury. Behav Brain Res. 2006;170(2):233-40.
31. Shiigi Y, Kaneto H. Facilitation of memory retrieval by pre-test morphine and its state dependency in the step-through type passive avoidance learning test in mice. Jpn J Pharmacol. 1990;54(1):79-81.
32. Bruins Slot LA, Colpaert FC. Opiate states of memory: receptor mechanisms. J Neurosci. 1999;19(23):10520-9.
33. Bruins Slot LA, Colpaert FC. Recall rendered dependent on an opiate state. Behav Neurosci. 1999;113(2):337-44.
34. Bruins Slot LA, Colpaert FC. A persistent opioid-addiction state of memory. Behav Pharmacol. 2003;14(2):167-71.
35. Patti CL, Kameda SR, Carvalho RC, Takatsu-Coleman AL, Lopez GB, Niigaki ST, et al. Effects of morphine on the plus-maze discriminative avoidance task: role of state-dependent learning. Psychopharmacology (Berl). 2006;184(1):1-12.
36. Ragozzino ME, Gold PE. Glucose injections into the medial septum reverse the effects of intraseptal morphine infusions on hippocampal acetylcholine output and memory. Neuroscience. 1995;68(4):981-8.
37. Niu L, Cao B, Zhu H, Mei B, Wang M, Yang Y, et al. Impaired in vivo synaptic plasticity in dentate gyrus and spatial memory in juvenile rats induced by prenatal morphine exposure. Hippocampus. 2009;19(7):649-57.

38. Homji NF, Vigorito M, Chang SL. Morphine-induced conditioned place preference and associated behavioural plasticity in HIV-1 transgenic rats. Int J Clin Exp Med. 2012;5(2):105-23.
39. Alcantara AA, Lim HY, Floyd CE, Garces J, Mendenhall JM, Lyons CL, et al. Cocaine- and morphine-induced synaptic plasticity in the nucleus accumbens. Synapse. 2011;65(4):309-20.
40. Andreescu CE, Milojkovic BA, Haasdijk ED, Kramer P, De Jong FH, Krust A, et al. Estradiol improves cerebellar memory formation by activating estrogen receptor beta. J Neurosci. 2007;27(40):10832-9.
41. Su J, Sripanidkulchai B, Sripanidkulchai K, Piyachaturawat P, Wara-Aswapati N. Effect of Curcuma comosa and estradiol on the spatial memory and hippocampal estrogen receptor in the post-training ovariectomized rats. J Nat Med. 2011;65(1):57-62.
42. Wiescholleck V, Manahan-Vaughan D. Persistent deficits in hippocampal synaptic plasticity accompany losses of hippocampus-dependent memory in a rodent model of psychosis. Front Integr Neurosci. 2013;7:12.
43. Manns JR, Hopkins RO, Reed JM, Kitchener EG, Squire LR. Recognition memory and the human hippocampus. Neuron. 2003;37(1):171-80.
44. Aitta-aho T, Moykkynen TP, Panhelainen AE, Vekovischeva OY, Backstrom P, Korpi ER. Importance of GluA1 subunit-containing AMPA glutamate receptors for morphine state-dependency. PLoS One. 2012;7(5):e38325.
45. Mariani RK, Mello CF, Rosa MM, Ceretta AP, Camera K, Rubin MA. Effect of naloxone and morphine on arcaine-induced state-dependent memory in rats. Psychopharmacology (Berl). 2011;215(3):483-91.
46. Slamberova R, Schindler CJ, Pometlova M, Urkuti C, Purow-Sokol JA, Vathy I. Prenatal morphine exposure differentially alters learning and memory in male and female rats. Physiol Behav. 2001;73(1-2):93-103.
47. Cui J, Wang Y, Dong Q, Wu S, Xiao X, Hu J, et al. Morphine protects against intracellular amyloid toxicity by inducing estradiol release and upregulation of Hsp70. J Neurosci. 2011;31(45):16227-40.
48. Nickelsen T, Lufkin EG, Riggs BL, Cox DA, Crook TH. Raloxifene hydrochloride, a selective estrogen receptor modulator: safety assessment of effects on cognitive function and mood in postmenopausal women. Psychoneuroendocrinology. 1999;24(1):115-28.
49. Neele SJ, Rombouts SA, Bierlaagh MA, Barkhof F, Scheltens P, Netelenbos JC. Raloxifene affects brain activation patterns in postmenopausal women during visual encoding. J Clin Endocrinol Metab. 2001;86(3):1422-4.
50. Yaffe K, Krueger K, Sarkar S, Grady D, Barrett-Connor E, Cox DA, et al. Cognitive function in postmenopausal women treated with raloxifene. N Engl J Med. 2001;344(16):1207-13.
51. Lacreuse A, Wilson ME, Herndon JG. Estradiol, but not raloxifene, improves aspects of spatial working memory in aged ovariectomized rhesus monkeys. Neurobiol Aging. 2002;23(4):589-600.
52. Audesirk T, Cabell L, Kern M, Audesirk G. beta-estradiol influences differentiation of hippocampal neurons in vitro through an estrogen receptor-mediated process. Neuroscience. 2003;121(4):927-34.
53. Haskell SG, Richardson ED. The effect of raloxifene on cognitive function in postmenopausal women: a randomized clinical trial. Conn Med. 2004;68(6):355-8.
54. O'Neill K, Chen S, Brinton RD. Impact of the selective estrogen receptor modulator, raloxifene, on neuronal survival and outgrowth following toxic insults associated with aging and Alzheimer's disease. Exp Neurol. 2004;185(1):63-80.

55. Goekoop R, Duschek EJ, Knol DL, Barkhof F, Netelenbos C, Scheltens P, et al. Raloxifene exposure enhances brain activation during memory performance in healthy elderly males; its possible relevance to behavior. Neuroimage. 2005;25(1):63-75.
56. Goekoop R, Barkhof F, Duschek EJ, Netelenbos C, Knol DL, Scheltens P, et al. Raloxifene treatment enhances brain activation during recognition of familiar items: a pharmacological fMRI study in healthy elderly males. Neuropsychopharmacology. 2006;31(7):1508-18.
57. Schilder CM, Linn SC, van Dam FS, Schagen SB. [The effect of hormone therapy on cognitive function in patients with breast cancer]. Ned Tijdschr Geneeskd. 2008;152(9):494-8.
58. Jacobsen DE, Samson MM, Emmelot-Vonk MH, Verhaar HJ. Raloxifene improves verbal memory in late postmenopausal women: a randomized, double-blind, placebo-controlled trial. Menopause. 2010;17(2):309-14.
59. Jacobsen DE, Melis RJ, Verhaar HJ, Olde Rikkert MG. Raloxifene and tibolone in elderly women: a randomized, double-blind, double-dummy, placebo-controlled trial. J Am Med Dir Assoc. 2012;13(2):189 e1-7.
60. Velazquez-Zamora DA, Garcia-Segura LM, Gonzalez-Burgos I. Effects of selective estrogen receptor modulators on allocentric working memory performance and on dendritic spines in medial prefrontal cortex pyramidal neurons of ovariectomized rats. Horm Behav. 2012;61(4):512-7.
61. Yang ZD, Yu J, Zhang Q. Effects of raloxifene on cognition, mental health, sleep and sexual function in menopausal women: a systematic review of randomized controlled trials. Maturitas. 2013;75(4):341-8.
62. Merchenthaler I, Funkhouser JM, Carver JM, Lundeen SG, Ghosh K, Winneker RC. The effect of estrogens and antiestrogens in a rat model for hot flush. Maturitas. 1998;30(3):307-16.
63. Alfinito PD, Chen X, Atherton J, Cosmi S, Deecher DC. ICI 182,780 penetrates brain and hypothalamic tissue and has functional effects in the brain after systemic dosing. Endocrinology. 2008;149(10):5219-26.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=582&id=32&aid=5358