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The Effect of Intrahippocampal Injection of Insulin-like Growth Factor-1 on Morphine-Induced Amnesia in Wistar Rats

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Abstract

Background: Morphine is wildly used as a painkiller in clinics, but causes several side effects such as hyperalgesia, depression and more notably amnesia. Here, we assumed that insulin-like Growth Factor-1 (IGF-1) as a peptide with widespread distribution of receptors in brain regions, especially hippocampus, might be capable to alleviate morphine-induced amnesia.

Methods: Thirty one male Wistar rats were divided into four groups including: morphine+saline, saline+saline, Morphine+IGF-1 and Saline+IGF-1 groups. The animals were cannulated in hippocampus using stereotaxic apparatus. IGF-1 (5 µg/ 1µL /rat) was intrahippocampally injected 30 minutes prior to morphine (10 mg/kg/i.p) injection, and then rats were trained in step-through passive avoidance task. First Latency time (FLT) to enter and total time spent (TTS) in dark were measured 1.5 and 24 hours later. Control group received the same volume of saline.

Results: The results showed that injection of 10 mg/kg morphine, compared with saline, significantly decreased FLT (p=0.001), but increased TTS (p=0.001) 24 hours after the training. Whereas, administration of IGF-1 compared with morphine, significantly increased FLT (p=0.001), but decreased TTS (p=0.001) assessed 24 hours after the training,

Conclusions: These findings indicate that administration of morphine disturbs passive avoidance learning and memory and injection of IGF-1, 30 minutes before morphine injection, prevents amnesia.

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Introduction

Morphine is an important opioid ligand used as a pain reliever because of its ability to activate opioid receptor in the central nervous system (1). However, it causes various side effects such as hyperalgesia, nausea, constipation, physical dependence, depression and amnesia (2).

It has been well recognized that the opioid system is involved in learning and memory functions (3). Both pre- and post-training administrations of morphine in hippocampus cause retention memory impairment in passive avoidance task (4, 5), in a dose- and time-dependent manner (6, 7). Convergent findings from humans and animals studies have shown that

passive avoidance paradigm depends on hippocampal function beside amygdala structure (8, 9). Hippocampus is a critical region to consolidate short term memory to long-term (10, 11).

Insulin-like Growth Factor-1 (IGF-1) is a polypeptide hormone structurally similar to insulin (12). IGF-1 is produced by all major CNS cell types particularly in the cortex, hippocampus, cerebellum and hypothalamus (13). Moreover, its receptors are found in the hippocampus, amygdala and parahippocampal gyrus (14). This peptide involves in expansion of neurons and neurogenesis (15), and is assumed to be capable in ameliorating cognitive disorders and neurodegeneration in adulthood (15, 16). Interestingly, it has been found that IGF-1 signaling reduces with age, and leads to neurodegenerative disorders (12).

Considering the fact that, morphine is widely used in clinic, and causes amnesia in patients, to find a strategy to combat amnestic effect of morphine has a great importance. Due to the positive effects of morphine on learning and memory formation, we assumed that IGF-1 might be capable to interfere with morphine- induced amnesia.

To evaluate this, we used step through passive avoidance task which is a one-trial emotional memory task combining fear conditioning with an instrumental response (17) suitable for pharmacological evaluation of associative memory.

Materials and methods

Animals and ethics statement

Thirty one male Wistar rats weighing 200-250 g were assigned randomly to the four groups (n=8): 1. morphine+saline, 2. saline+saline, 3. morphine+IGF-1, 4. saline+IGF-1 (n=7). All animals were housed three per cage at standard conditions (22 ± 2°C, 12 h light-dark cycle) and they had free access to food and water. All experiments were conducted in accordance with the National Institutes of Health guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978) modified by the Ethics Committee of Guilan University of Medical Sciences.

Surgery and drug administration

The animals were anesthetized intraperitoneally with a mixture of ketamine and xylazine (75 and 5 mg/kg, respectively). The rats were bilaterally cannulated in the hippocampal CA1 area (Figure 1), using stereotaxic apparatus (Stoelting, Chicago, USA) according to the coordination of hippocampus: AP: − 3mm, L: ± 2mm and V: − 2.8mm (18).

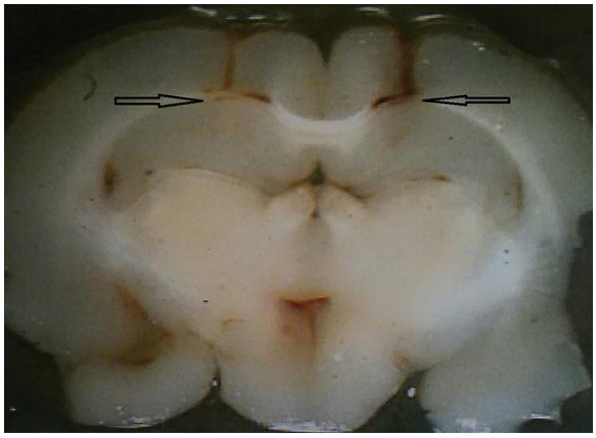

Figure1. Photomicrograph of rat brain section indicating the site of injection into the hippocampus

Morphine (Darupakhsh Co., Iran) and IGF-1 (recombinant IGF-1, Chemicon International, Temecula, USA) were dissolved in 0.9% saline. Based on the assigned groups, the animals received a single dose of 10 mg/kg morphine (4) or saline intraperitoneally, 30 minutes before training. In the other

two groups, animals received bilateral infusion of saline or IGF-1(5 µg, 1 µL/ rat) intrahippocampally (4, 19-21), 30 minutes before morphine treatment. IGF-1 was gently injected at 9^{AM} through a polyethylene tube attached to a 10 µL Hamilton syringe on each side during 5 minutes, and then the needle (27-gauge) was kept in the cannula (22-gauge) for 2 minutes.

Step-Through passive avoidance

The apparatus consisted of two compartments (bright and dark, 20×20×30 cm) separated by a guillotine door (7×9 cm). The floor of dark compartment was covered with electrified bars. Behavioral procedures started 30 minutes after morphine treatment. The rats were trained in step-through inhibitory avoidance task, in a way that each rat was individually placed in the bright compartment and the door was opened after 20 seconds. When the animals entered to the dark compartment, electric shocks (0.5 mA) were given continuously for 5 seconds. The retention memory was measured 1.5 and 24 hours following the training. First Latency time (FLT) and total time spent (TTS) were recorded in three minutes (5).

Statistical analysis

Normality of variables was estimated by Kolmogorov-Smirnov and Shapiro-Wilk test. When the values of p were < 0.05, data were analyzed using the nonparametric Kruskal Wallis test. Data were expressed as mean ± standard error (mean ± SE) and values of $p < 0.05$ were considered statistically significant (SPSS 22 software, Chicago, USA).

Results

Following the administration of saline, morphine and IGF-1 according to the treatment group, the rats were trained in passive avoidance task and retention memory was assessed 1.5 and 24 hours after the acquisition process.

As figure 2 shows, intraperitoneal injection of 10 mg/kg morphine compared with the saline group significantly decreased FLT (p=0.003), but increased TTS (p=0.001), 1.5 hours after the training (a, b). Also, 24 hours after the training, the morphine receiving group compared with the saline group showed significant reduction of FLT, but elevation of TTS (p=0.001, c, d).

Administration of IGF-1 30 minutes before morphine in the hippocampal CA1 area caused significant difference in FLT (p=0.005) and TTS (p=0.001) compared with the morphine group 1.5 hours after the training (Figure 2, a and b). In addition, figures 2c and 2d show significant elevation in FLT, but reduction in TTS in the morphine+IGF-1 group compared with the morphine+saline group with the values of p=0.001 and p=0.001 respectively, 24 hours after the training.

However, passive avoidance learning and memory did not significantly change between saline+IGF-1 and saline+saline groups (Figure 2).

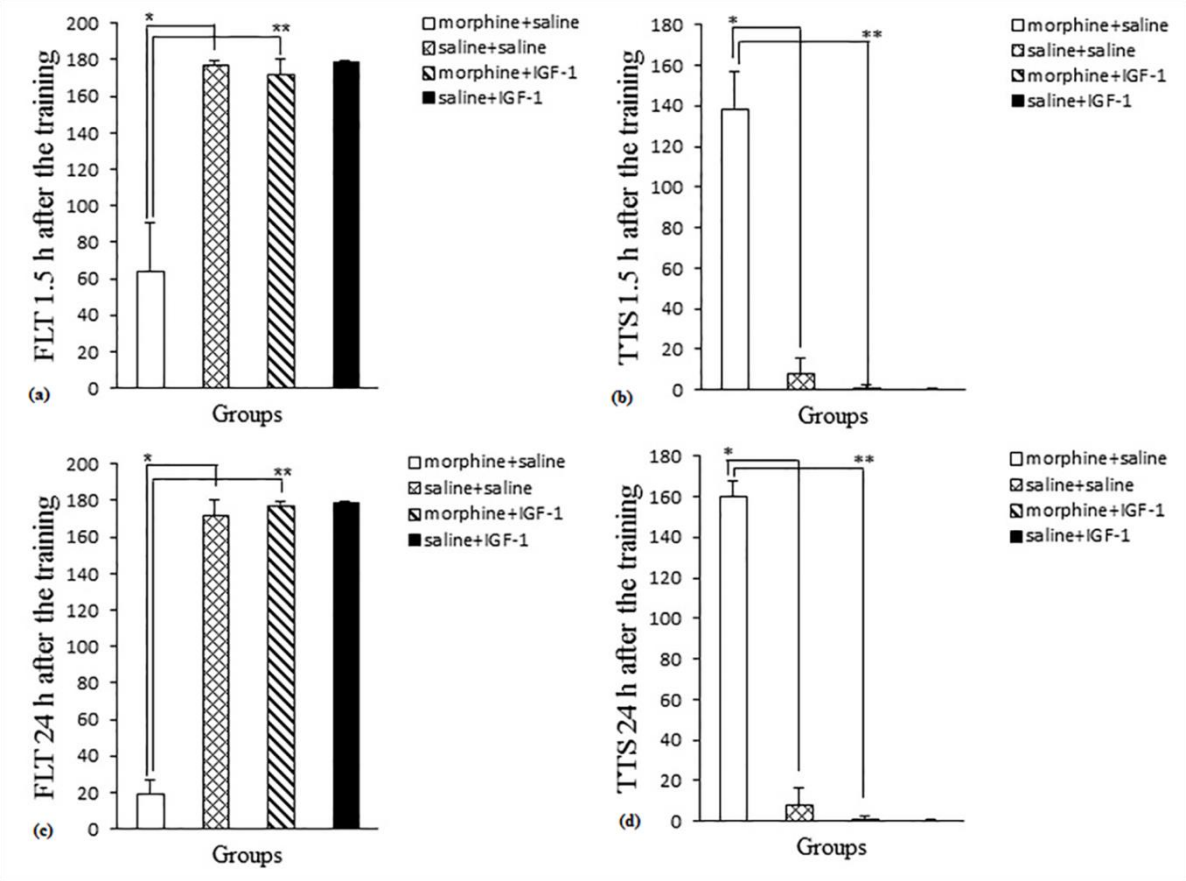

Figure 2. The effect of IGF-1 treatment prior to morphine injection on amnesia

FLT and TTS in the four groups 1.5 h and 24 h after the training expressed as mean ± SE. Nonparametric Kruskal Wallis test was used. *:$p < 0.05$ compared with the saline+saline group, **:$p < 0.05$ compared with the morphine+saline group, FLT: First latency time, TTS: Total time spent

Discussion

Our findings are in agreement with the results of previous studies (4, 7, 22) and showed that pre-training infusion of morphine disturbs passive avoidance learning and memory. Also, morphine administration in hippocampus has been reported to induce cognitive deficits in various memory tasks (23, 24). It has been suggested that the effects of morphine on learning and memory are mainly mediated via μ-opioid receptors in both hippocampus (25) and central amygdala (26) through blocking voltage-gated calcium (Ca^{2+}) channels, and then efflux of potassium (24).

Moreover, morphine is capable to modify the release of neurotransmitters (26), and metabolism of glucose (27, 28).

In addition, the present study showed that, IGF-1 pre-treatment before acquisition of learning rescues memory impairments in amnesia induced by morphine. This finding confirms the reported positive effects of IGF-1 on neural

plasticity and neurogenesis (15,16), and aging-induced memory impairment (29).

Since we evaluated both short term and long term memory, the mechanism responsible for improvement of memory, might be the modulatory effect of IGF-1 on synaptic plasticity and neurotransmission (30, 31) via AMPA receptors and voltage-gated Ca^{2+} channel (32). Also, IGF-1 regulates glucose signaling via glucose transporters expression (33), and increases insulin signaling which is pivotal for brain function.

Moreover, it cannot exclude the possibility that, IGF-1 has potent actions on axonal development and synapse formation through elevating phosphoinositide 3/Akt kinases (34).

Regarding the fact that morphine induces glucose metabolism (25), and IGF-1 regulates glucose signaling (33), we can postulate that restoring memory in amnestic animals might be related to glucose utilization beside neurotransmission mechanisms.

Conclusion

In conclusion, these results suggest that IGF-1 restores associative learning and memory in amnesia induced by morphine.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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