Original Article

Effect of forced treadmill exercise and blocking of opioid receptors with naloxone on memory in male rats

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Abstract

Background: The forced treadmill running can influence the opioid contents of the brain, through both effects of exercise and the effects of stress caused by coercion. Since opioids can cause negative effects on brain functions, this study aimed to evaluate the effect of forced treadmill exercise and blocking of opioid receptors with naloxone on memory in male rats.

Materials and Methods: Experimental groups were the control, the exercise, the naloxone, and the naloxone exercise. The exercise program was treadmill running at 22 m/min at 0° inclination for 50 min/day, 6 days/week, for 4 weeks. Naloxone (1 mg/kg) was injected 5 min before the treadmill running. Morris water maze and passive avoidance learning tests were used for evaluation of memory. Acquisition phase of both tests was performed before interventions, and memory was evaluated 1-day and 1-week after the last session of exercise and treatments.

Results: Our data showed that forced exercise impaired performance in passive avoidance learning test ($P < 0.05$ and $P < 0.01$, 1-day, and 1-week after the last session of exercise and treatments, respectively). Spatial memory was only impaired after 1-week in the exercise group. Naloxone had no significant effect on memory in the control group. However, it improved memory in the exercise group, as there was no significant difference between the control and the naloxone exercise in both tests.

Conclusion: The data correspond to the possibility that opioidergic system may have mediatory roles in exercise-induced responses in forced exercise. These roles are likely harmful for memory.

Key Words: Forced treadmill running, naloxone, memory, rat

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INTRODUCTION

It is reported that exercise and physical activity increase the release of endogenous opioid peptides in the brain and the exercise-induced euphoria is related to opioidergic mechanisms.[1] Animal studies and clinical findings showed that long-term regular exercise can activate the central opioid system and stimulate the release of endogenous opioid peptides and increase the pain threshold in both humans and animals.[2,3] Therefore, the release of opioids during exercise may be responsible for some of the effects of exercise on the nervous system. These opioids can have the same effects with morphine and other opioid receptor agonists.[4]

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Studies have shown conflicting effects of opiates on cognitive processes such as learning and memory. Some studies have shown that stimulation of opioid receptors can have favorable effects on learning and memory while others have shown that inhibition of opioid receptors can have favorable effects on learning and memory.[5,6] It has been shown that the use of morphine prior to exercise in rat modulate fatigue in forced treadmill exercise and strengthen spatial learning and memory performance. Exercise boosts morphine's effect by increasing the release of endogenous endorphins.[7] However, other studies showed the negative effects of opiates on cognitive behaviors[6] and impairment of learning and memory.[8-10] It was reported that this morphine-induced learning and memory impairments can be prevented by naloxone (an opioid antagonist).[11,12]

In both human and animal studies, inconsistent effects of exercise on learning and memory have been shown. This is perceivable in complex behavioral tests as no change or impairment of learning. Some studies have shown that treadmill exercise, a form of forced exercise, had no negative effects on the level of apoptosis in the hippocampal dentate gyrus, but increases cell proliferation and improves learning and memory.[13-15] However, some studies have not shown these desired effects and in intact subjects, treadmill exercise had no favorable effects.[16,17] It was also found that regular physical activity could not improve and affect spatial learning and memory in the middle-aged and elderly ones.[18] This variation in the results may be related to differences in the protocol of exercise (voluntary vs. forced), the intensity, and duration of exercise.[19] Forced treadmill exercise can cause some stress responses because in this type of activity, time, duration, and intensity of exercise is determined by the experimenter and the animal is forced to run by a mild electric shock.[20] Thus, the forced exercises may be having different effects on neuronal functions in different situations, and this may be partly due to the stress.

Studies have shown that stress can affect the secretion of opioids in the brain, and an important part of the stress responses can mediate, modulate, and regulate by the endogenous opiate system.[21-23] Because forced treadmill exercise could possibly affect the secretion of brain opioids through both the exercise and the stress-induced by this type of exercise, therefore, the aim of this study was to evaluate the mediatory role of endogenous opioid system on the effect of forced treadmill running on avoidance and spatial memory, by blocking the opioid receptors, with naloxone, during running.

MATERIALS AND METHODS

Male Wistar rats (180–220 g) were housed four per cage and maintained on a 12 h light dark cycle in an air conditioned constant temperature (23 ± 1°C) room, with food and water made available *ad libitum*. The Ethic Committee for Animal Experiments at Isfahan University approved by the study, and all experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996.

Animals were divided into four groups: The control, the exercise, the naloxone, and the naloxone exercise (n = 10 for each experimental group).

Rats in the control exercise and naloxone exercise groups were subjected to run at the speed of 22 m/min for 50 min daily (6 days a week), for 4 weeks at 0° of inclination. To familiarize, animals were left on the treadmill for 50 min once a day for two consecutive days without operation of the treadmill, then from the 3rd day onward, the treadmill was switched on and the speed increased from 5 to 22 m/min and the duration increased from 10 to 50 min over the course of 5 days. Electric shocks were used sparingly to motivate the animal to run. From week 2 onward, after warm-up, speed, and duration were kept constant at 22 m/min and 50 min/run. The nonrunners groups were put on the treadmill without running for the same duration as the runners.

Naloxone (1 mg/kg; Darou pakhsh co., Iran)[24] was injected intraperitoneally 5 min before treadmill running.

Acquisition phases of Morris water maze (MWM) and passive avoidance learning tests were conducted before starting the exercise protocol and receiving naloxone. One day and 1-week after the last session of treatment and exercise retention phases of the tests were performed.

Passive avoidance learning test

The apparatus consists of two separate chambers connected through a guillotine door. One chamber was illuminated while the other was dark. The floor of both chambers consists of steel grids, used to deliver electric shocks. In the acquisition trail, 1-day before exercise and treatments, each rat was placed in the illuminated chamber while its back was to the guillotine door. After 30 s of habituation, the guillotine door separating the illuminated and dark chambers were opened and the initial latency to enter the dark

chamber was recorded. The guillotine door was closed immediately after the rat enters the dark chamber, and an electric foot shock (75 V, 0.2 mA, 50 Hz) was delivered to the floor grids for 3 s. Then, the rat was removed from the dark chamber and returned to its cage. One day and 1-week after the last session of exercise and treatment, retention latency time to enter the dark chamber was taken in the same way as in the acquisition trail, but the foot shock was not delivered, and the latency time was recorded up to a maximum of 600 s.

Morris water maze test

The circular tank (180 cm in diameter) was filled with water (22°C ± 2°C) made opaque and was surrounded by a variety of extra-maze cues. The tank was divided into four quadrants, and four start positions were located at the interactions of the quadrants. Data were recorded using custom software (Radiab1). Twenty-four hours before water maze testing, all rats were habituated to the water and apparatus. In the spatial acquisition phase, the rats learned to find a submerged platform using extra-maze cues. A transparent lucite platform (10 cm) was submerged 2 cm underneath the water in the South-East quadrant of the tank, where it remained for all spatial trials. Each rat participated in 16 trials, which were organized into a daily block of four trials (one trial/start position within a block) for four consecutive days prior to the start of exercise and treatment. For each trial, the rat was given a maximum time of 60 s to locate the platform, after which the rat remained there for 30 s; if the rat did not locate the platform within 60 s, it was guided to it by the experimenter. The next trial started immediately after removal of rat from the platform. Escape latencies (s) was recorded.[25] One day and 1-week after the last session of exercise and treatment in the retention phase, a 60 s probe trial was conducted to examine how well the rats had learned the exact location of the platform. During this trial, the platform was removed from the tank. The swim time was measured inside a circular area (70 cm diameter) around the center of the platform, and the number of crossing the exact location that previously the plat was located (plat crossing) was counted. To test possible deficits in sensorimotor processes, rats were tested in the water maze with a visible platform after probe trial.[17]

Statistical analysis

Data were analyzed using the SPSS 21 for Windows (IBM Corporation). The data were analyzed statistically by two-way ANOVA followed by Tukey *post-hoc* for between-subjects differences and within effects, across the blocks in the spatial acquisition phase of MWM; and the swim time from probe trial of MWM and the data from passive avoidance learning were analyzed by one-way ANOVA followed by Tukey; and the number of plat crossing from probe trial of MWM was analyzed by Kruskal–Wallis test (nonparametric ANOVA) and Dunn's multiple comparisons for posttest. The significant level was set at $P < 0.05$. Results are expressed as a mean ± standard error of the mean.

RESULTS

Passive avoidance learning test

In the acquisition trial, the mean initial latencies were same in all groups. One day and 1-week after the last session of exercise and treatment, step-through latency showed that forced exercise significantly impaired memory with respect to the control group ($P < 0.05$, $P < 0.01$, respectively). The naloxone had no significant effect on memory in the control rats. However, in the naloxone exercise group, the latency was increased, and no significant differences were observed compared to control group at the 1-day and 1-week [Figure 1a and b].

Morris water maze test

All rats showed a reduction in escape latencies (block effect, $F(3,102) = 43.49$, $P < 0.001$); [Figure 2] across the blocks of trials, indicating spatial acquisition. The pattern of reduction in escape latencies across the blocks had no significant differences between the

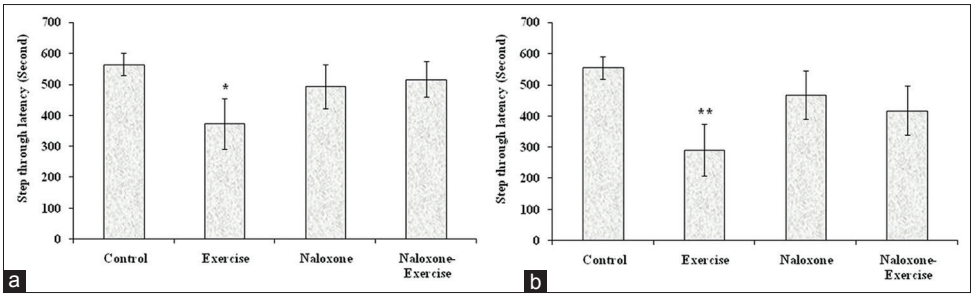

Figure 1: Effects of naloxone and forced treadmill running on step-through latency in passive avoidance test, 1-day (a) and 1-week (b) after the last session of exercise and treatment. Data are expressed as a mean ± standard error of the mean. *$P < 0.05$ and **$P < 0.01$ with respect to the control group (n = 9–10)

groups (GROUP*BLOCK effect interaction, F (9,102) = 1.12, P = 0.36); [Figure 2]. Test of between-subject effects did not show any significant difference between the groups.

For the results of probe trial 1-day after the last session of exercise and treatment, as measured by the mean time spent inside a circular area (70 cm diameter) [Figure 3a] and the number of plat crossing [Figure 3b], there were no significant differences between the experimental groups.

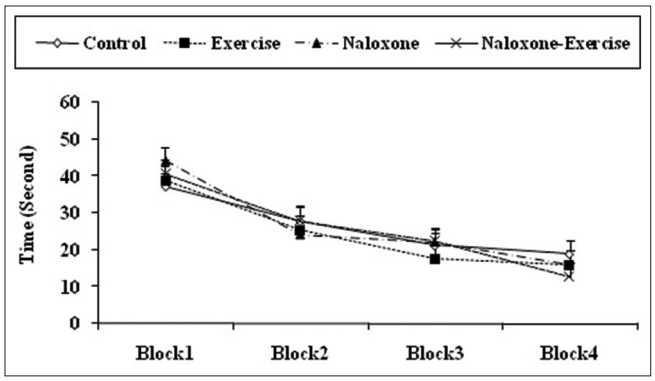

Figure 2: Effects of naloxone and forced treadmill running on the escape latency at different blocks to reach the platform during the spatial acquisition of Morris water maze test in rats. Each point represents mean ± standard error of the mean of four swims. Lower numbers indicate better performance (n = 9–10)

One week after the last session of exercise and treatment, the mean time spent inside a circular area (70 cm diameter) was same between the groups [Figure 4a]. The number of plat crossing was lower in the exercise group with respect to the control group (P < 0.05); [Figure 4b]. However, there were no significant differences between the other groups.

DISCUSSION

The results showed that forced treadmill exercise impairs memory. Although naloxone could not affect memory in intact rats, but the blockage of opioids receptors by naloxone during forced exercise prevented the impairments.

Although some studies have shown favorable effects of exercise on the central nervous system,[26-28] but according to the present results, forced exercise can cause some damage or is ineffective.[29] As mentioned above, forced treadmill running can be somewhat stressful, because in this type of exercise, all aspects of exercise are determined by the examiner and the animal is forced to run by a mild electric shock at the back.[20] Chronic stress due to coercion and applying electrical shocks in treadmill running can lead to enhancement of plasma cortisol[30] and can damage learning and memory pathways in the brain.

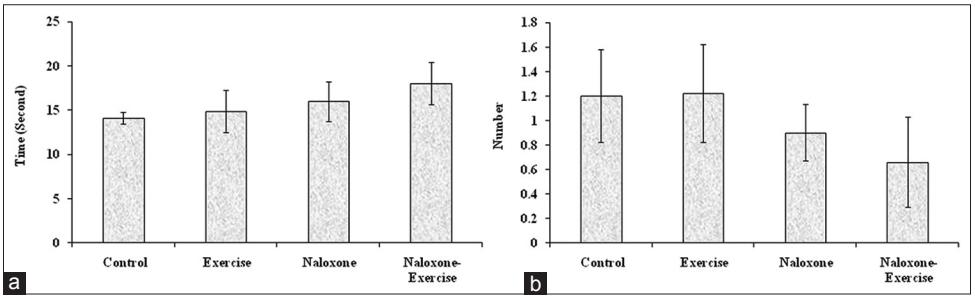

Figure 3: Effects of naloxone and forced treadmill running on performance during the probe trial 1-day after the last session of exercise and treatment, as measured by the mean time spent inside a circular (70 cm diameter) around the center of platform (a) and the number of crossing the exact location that previously the plat was located (plat crossing). (b) Data are expressed as mean ± standard error of the mean (n = 9–10)

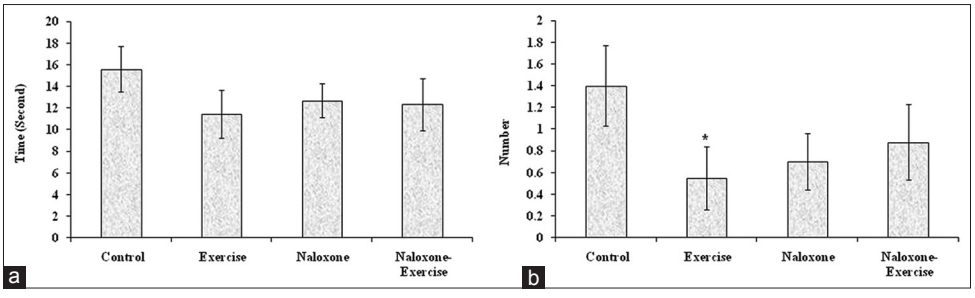

Figure 4: Effects of naloxone and forced treadmill running on performance during the probe trial 1-week after the last session of exercise and treatment, as measured by the mean time spent inside a circular (70 cm diameter) around the center of platform (a) and the number of crossing the exact location that previously the plat was located (plat crossing). (b) Data are expressed as a mean ± standard error of the mean. *P < 0.05 with respect to the control group (n = 9–10)

The hippocampus is a part of the limbic system, which is involved in learning and memory.[31,32] Studies have shown that this region is damaged after repeated stress.[33] Stress increases the adrenal steroids, and these steroids affect the hippocampus and inhibit proliferation of granular cells in dentate gyrus.[34-36] It has been demonstrated that adrenal steroids can cause deformation of dendrites in the hippocampus and damage to cognitive functions such as learning and memory.[36,37] However, some studies have shown favorable effects of forced exercise on learning and memory.[38] These differences may be due to differences in the intensity of exercise, differences of race and age of animals, which are known influencing factors.[39]

As a second result, we observed that naloxone had no significant effects on memory in intact rats. Studies have shown different results from the effects of naloxone on the cognitive process. Some studies have been reported that naloxone impaired avoidance learning but had no effect on spatial learning.[40] It was also reported that intravenous administration of high doses of naloxone damages memory[41] and increases anxiety in rats.[42] However, in this study, when naloxone was injected before exercise, it can prevent some of the damages caused by forced exercise.

Although opioids that are released during exercise can have favorable impact on the nervous system,[1,43] but in forced treadmill running, probably stress, and exercise can increase the release of opioids in the brain much more and provide similar effects as morphine. Numerous reports about the negative effects of morphine on learning and memory are presented.[44] It has been shown that acute and sudden use of morphine impair the process of learning and memory, but for the long-term and chronic use of morphine contradictory results have been reported.[8,9] Studies have shown the involvement of opioid system in regulating the fear and anxiety.[45] Acute stress increases the release of opioids in the rats.[46] High levels of endogenous opioids in the brain can stimulate hypothalamic-pituitary-adrenal axis similar to morphine[45] and can stimulate the release of adrenal steroids as higher than normal, which could strengthen the effects of stress. In addition, released opiates during running may induce analgesia[4] and raise tolerance to pain and shock in rats. So the rats resist for running, and they get more electric shocks. Consequently, less exercise and more stress damage memory. Thus, blockage of opioid receptors during exercise could counteract the effects of high levels of opioids and reduce the damages by reducing the effects on the hypothalamic-pituitary-adrenal axis.

The results suggest that opioidergic system may have mediatory roles in exercise-induced responses in forced exercise. These roles are likely harmful for the cognitive functions. Blockage of opioid receptors during exercise can reduce the harmful responses that are resulted from over release of opioids and can prevent some of the damages.

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Conflicts of interest
There are no conflicts of interest.

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