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Research report

Central β-adrenergic receptors play an important role in the enhancing effect of voluntary exercise on learning and memory in rat

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ABSTRACT

The beneficial effects of physical activity and exercise on brain functions such as improvement in learning and memory are well documented. The aim of this study was to examine the role of the β-adrenergic system in voluntary exercise-induced enhancement of learning and memory in rat. In order to block the β-adrenergic receptors, the animals were received propranolol (a β-blocker), or nadolol (a peripherally acting β-blocker) before each night of five consecutive nights of exercise. Then their learning and memory were tested on the water maze task using a two-trials-per-day for 5 consecutive days. A probe trial was performed 2 days after the last training day. Our results showed that propranolol, but not nadolol reversed the exercise-induced improvement in learning and memory in rat. Our findings indicate that central β-adrenergic receptors play an important role in mediating the beneficial effects of voluntary exercise on learning and memory.

1. Introduction

Several reports have studied the useful effects of physical activity and exercise on brain functions such as enhancement in learning and memory [14,20], cognitive function [21], neurogenesis [32] and recovery from brain injury [16]. It has been observed that exercise can improve the performance of experimental animals in tests of spatial learning [32,33]. A number of mechanisms such as noradrenergic and serotonergic neurotransmission [15,19], brain derived neurotrophic factor (BDNF) receptor activation [29], insulin-like growth factor I receptor activation [7,10] and vascular endothelial growth factor receptor activation [13] have been suggested as the mediators of exercise effects on brain.

It is well established that noradrenergic transmission is critical for various forms of learning and memory in rats. Administration of epinephrine and its agonist after training increases memory performance of aversively motivated learning [17]. Epinephrine effects on memory consolidation is blocked by propranolol, a β-blocker which crosses the blood–brain barrier easily, as well as sotalol, another β-blocker that does not readily enter the brain [18]. These findings suggest that both peripheral and central β-adrenergic receptors mediate the effects of epinephrine on memory storage. Also, the finding that epinephrine effects on memory storage are blocked by propranolol infused into the basolateral amagdala (BLA) [23], suggests that epinephrine effects, at least in part, are mediated by β-adrenergic receptors located in the BLA.

Recent studies have revealed the important role of the noradrenergic system in mediating the effects of exercise on the brain [15,19]. Wheel and treadmill running was found to increase the nor-epinephrine levels in several brain regions such as hippocampus [19]. Also, it has been shown that an intact noradrenergic system may be crucial for the observed ability of exercise to enhance hippocampal BDNF mRNA expression [15]. Moreover, very recently we have found that lesion of the noradrenergic system eliminated the enhancing effects of maternal exercise on spatial learning and memory in rat pups, indicating a specific role for the noradrenergic system in mediating the enhancing effects of maternal exercise on offspring cognitive functions [1].

The aim of the present work was to examine whether exercise enhances spatial learning and memory via the β-adrenergic system. We used a water maze (WM) task to study the possible relationship between voluntary exercise-induced noradrenergic activity and spatial learning and memory enhancement. The role of the β-adrenergic system in the beneficial effects of exercise on spatial cognitive functions was determined by systemic administrations of the β-adrenergic receptors antagonists propranolol and nadolol.

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2. Materials and methods

2.1. Animals

Adult male Wistar rats (220 ± 10 g) were obtained from breeding colony of Semnan University of Medical Sciences, Semnan, Iran. All rats were individually housed in cages in a 12-h light/dark cycle at 22–24 °C, with food and water *ad libitum*. All procedures were conducted in agreement with the National Institutes of Health Guide for care and use of laboratory animals. Also, in each experiment care was taken to use the minimum number of the animals.

2.2. Exercise paradigm

Each of the exercise rats was given access to a running wheel (diameter = 34.5 cm, width = 9.5 cm) that was freely rotated against a resistance of 100 g. Each wheel was equipped with a magnetic switch which was connected to a separate counter located outside of the animal house, so that it was possible to check the revolutions without disturbing the exercising animals. The distance (D) of running for each animal was calculated (as meter) as follows:

$$D = N \times 2\pi r$$

where N is the number of the wheel revolutions and r is the radius of the wheel. The revolutions of each wheel were recorded every day at 6 a.m. The sedentary rats were confined to the similar cages with no access to the wheels. The exercise groups were exposed to 5 days of exercise after which the running wheels were removed from their cages and their learning and memory were tested on the WM task. During the WM experiment, all groups were exposed to the same conditions as described above in the same animal house.

2.3. Drugs

Propranolol (PROP, Sigma–Aldrich) and nadolol (NAD, Sigma–Aldrich) were dissolved in physiological saline and administered (IP) at a dose of 10 and 20 mg/kg at volume of 2 ml/kg, respectively. Control animals received an equivalent volume of saline. We selected the doses of the drugs on the basis of pilot and other studies [27]. PROP and NAD have similar potency as β-receptor antagonists. Both drugs are non-selective β-adrenergic receptor antagonists. PROP readily passes the blood–brain barrier and thus acts on both peripheral as well as central β-adrenergic receptors. NAD has limited ability to cross the blood–brain barrier and thus acts primarily at peripheral β-adrenergic receptors [6,12,24]. We injected NAD at twice the dose of PROP. It was to make sure that any lack of effect of NAD on exercise-induced enhancement of learning and memory was not merely due to lesser occupancy of peripheral β-receptors by NAD. All rats received the injections (saline or antagonists) during the 5 nights running period once per day at 6 p.m.

2.4. Testing of spatial learning and memory in the WM

For the WM test, we used a swimming pool (140 cm diameter, 60 cm height) which was divided into four quadrants. The quadrant housing the escape platform (11 cm diameter) was designated as the target zone, such that the escape platform was fixed in a permanent position 2 cm under the water surface during the course of the WM procedures. The other three quadrants were designated as left, right and opposite to the target zone. The water was kept at a steady 22 ± 2 °C. Spatial reference cues around the pool were maintained in their fixed positions through out the duration of the WM experiments. A camera suspended above the maze was wired to an automated tracking system (EthoVision, Version 3.1, The Netherland). The WM protocol which was used in this report has been described elsewhere [1,2]. This is a stringent two-trials-per-day, 5-day WM training protocol, identified as a good discriminative test for the effect of exercise on learning and memory [10,33]. Each rat was given two-trials-per-day for 5 consecutive days. The animals were placed into the pool facing the wall from one of four equally spaced start locations. Each release point was randomly altered every trial. Each trial lasted until the rat found the platform or for a maximum duration of 60 s. Animals who failed to find the platform within the allocated time were gently guided to the platform. At the end of each trial, animals were allowed to stay on the platform for 20 s. The escape latency (platform search time) for each trial was recorded. After the last trial, the animal was towel dried and returned to the home cage with no access to a running wheel. A spatial probe test was performed 2 days after the last acquisition trial, during which the platform was removed. Rats were allowed to swim for 60 s during which latency to reach the platform location and time spent swimming in within a zone, 20 cm in radius centered either the original training location (target zone) or an equivalent location in the opposite quadrant (opposite zone) were recorded. Velocity of each animal also was calculated. Analysis of time spent within a specified radius (zone) centered on the platform location is a more sensitive measure of water maze probe test performance [26].

2.5. Statistical analysis

Statistical analyses of the learning and memory data were performed using an analysis of variance (ANOVA) followed by a Bonferroni-protected *post hoc* t-test.

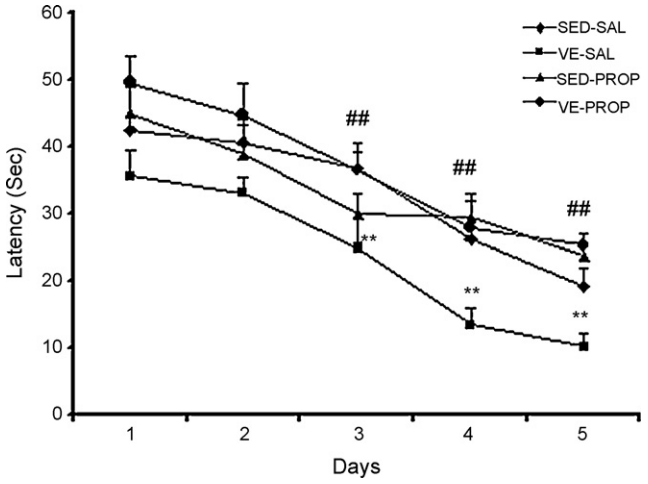

Fig. 1. Effect of blocking the β-adrenergic receptors by propranolol during exercise on learning acquisition as measured by the WM task. Exercise improved learning ability as exercise animals took significantly less time to learn the location of the platform than their sedentary control counterparts. Blocking the β-adrenergic receptors by propranolol receptors during exercise suppressed the exercise-induced learning enhancement in rats. * represents the significant difference between voluntary exercise-saline (VE-SAL) with and sedentary-saline (SED-SAL) or SED-propranolol (SED-PROP) groups. # represents the significant difference between VE-propranolol (VE-PROP) and VE-SAL groups. Data are expressed as the mean ± S.E.M.; $^{**}P < 0.01$, $^{\#\#}P < 0.01$.

Running distances were analyzed using a student t-test. Statistical differences were considered significant when $P < 0.05$.

2.6. Experimental protocol

2.6.1. Experiment 1

The aim of experiment1 was to examine the effect of PROP on exercise-induced enhancement of learning and memory.

2.6.1.1. Methods. Rats were randomly assigned to four groups ($n = 10$ animals per group): sedentary-saline (SED-SAL), voluntary exercise-saline (VE-SAL), sedentary-PROP (SED-PROP), and voluntary exercise-PROP (VE-PROP). The exercise rats were given 5 days of voluntary exercise according to procedure described above. All rats received the injections (saline or PROP) once per day (each day at 6 pm) prior to 5 nights running period.

2.6.1.2. Results. Student t-test on the cumulative running distances (m) of voluntary exercise for exercising groups (VE-SAL: 9727 ± 1159; VE-PROP: 11,642 ± 899) revealed no significant differences among groups ($t_{18} = -1.26$, $P = 0.22$). Acquisition data of the experimental groups during 5 days training in the water maze are illustrated in Fig. 1. A two-way ANOVA on the escape latencies showed significant groups ($F_{3,36} = 9.49$, $P < 0.001$) and days effects ($F_{4,36} = 43.04$, $P < 0.0001$). Between group comparisons indicated that escape latencies of VE-SAL group were significantly lower than that of SED-SAL group on all days ($P < 0.01$, $P < 0.01$, $P < 0.01$ and $P < 0.05$ for days of 2, 3, 4 and 5, respectively) except in day 1. The escape latency of VE-PROP group was significantly longer than that of VE-SAL group in day 3 ($P < 0.01$), day 4 ($P < 0.01$) and day 5 ($P < 0.01$). The differences between escape latencies between VE-SAL and SED-PROP groups was significant in days 3–5 ($P < 0.01$ in all cases). No significant differences were found between groups of SED-SAL and SED-PROP. These findings indicate that the enhancing effects of exercise on learning acquisition were blocked by propranolol.

Data from memory retention test is shown in Fig. 2. A two-way ANOVA on the platform location latency data indicated a significant effects of exercise ($F_{1,36} = 11.06$, $P = 0.002$), lack of significant effects of PROP ($F_{1,36} = 0.06$, $P = 0.8$), but a significant interaction between exercise and PROP ($F_{1,36} = 5.8$, $P = 0.02$). *Post hoc* comparisons indicated that the platform location latency of VE-SAL group is significantly lower than that of SED-SAL ($P < 0.01$), SED-PROP ($P < 0.05$) and VE-PROP ($P < 0.05$) groups. A two-way ANOVA on the time spent in zones showed a significant groups × zones effect ($F_{3,72} = 3.14$, $P = 0.02$). Between group comparisons indicated that the VE-SAL group spent more time in the target zone than the SED-SAL ($P < 0.01$), SED-PROP ($P < 0.01$), and VE-PROP ($P < 0.05$) groups. Also, VE-PROP group spent significantly more time in the target zone than the SED-PROP group ($P < 0.05$). These findings indicate that PROP was able to block, partially, an enhancement of memory retention by exercise. This suggests that other transmitter systems are engaged as well in the beneficial impact of exercise.