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Research report

The glucocorticoid system is required for the voluntary exercise-induced enhancement of learning and memory in rats

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ABSTRACT

Although it is well established that voluntary exercise can improve cognitive functions, the underlying mechanisms are largely unknown. Glucocorticoids play an important role in learning and memory functions. This study addressed whether the glucocorticoid system would play a role in the exercise-induced enhancement of learning and memory. Intact rats or those that were either adrenalectomized or daily given the corticosterone-synthesis inhibitor metyrapone were allowed to freely exercise in a running wheel for 10 days. Control animals were kept sedentary for this period. After this period, they were trained and tested on a water-maze spatial task using three-trial per day for 5 consecutive days, succeeded by a probe trial two days later. Exercise increased plasma corticosterone levels, as assessed after this 10-day period. Both adrenalectomy and metyrapone slightly reduced running-wheel activity. Adrenalectomy reduced the plasma corticosterone levels to almost zero whereas metyrapone selectively blocked the exercise-induced increase in corticosterone levels. Exercise significantly improved performance during both training and retention of the water-maze task whereas this effect was absent in both adrenalectomized and metyrapone-treated rats. These findings indicate that the glucocorticoid system play a crucial role in the beneficial effects of voluntary exercise on cognitive functions in rats.

1. Introduction

Voluntary exercise has been shown to facilitate learning and memory in a wide variety of hippocampus-dependent tasks such as the water maze, radial-arm maze, contextual fear conditioning, and passive avoidance conditioning in animals [2,3,34,39]. Voluntary exercise has also been shown to recover functional loss after central nervous system damage [17], and to reduce cognitive decline during aging [20]. Although the biological mechanisms that underlie such beneficial effects are still to be completely elucidated, possible mechanisms are an enhancement of neurogenesis, synaptic plasticity and brain-derived neurotrophic factor levels in the hippocampus [38,39], activation of noradrenergic and serotonergic systems [13,15,19] or increased neuronal uptake of circulating insulin like growth factor-I [6,10].

Physical exercise is known to correlate with striking metabolic changes and elicits neuro-endocrine responses, including that of the hypothalamic–pituitary–adrenal (HPA) axis [21]. Recent studies have shown that long-term voluntary wheel running, like other stressors, activates the HPA axis and results in glucocorticoid production [11,16,24,26]. Voluntary exercise appears to exert its stimulatory effects at multiple levels of the HPA axis [35]. In addition to supporting physical activity via metabolic modulation [8], glucocorticoids also have complex effects on the central nervous system, including effects on learning and memory. In fact, extensive evidence from animals and humans studies indicates that the administration of low doses of glucocorticoid hormones enhances memory for stressful or emotionally arousing events. These enhancing effects depend on the integrity of the amygdala noradrenergic system [31].

As voluntary exercise activates the HPA axis resulting in glucocorticoid production, this study investigated whether the glucocorticoid system might play a role in the exercise-induced enhancement of learning and memory. We used a water maze (WM) task to study the possible interaction between the voluntary exercise-induced glucocorticoid activity and the spatial learning and memory enhancement. Two experimental approaches were used to reduce the concentration of circulating corticos-

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terone; pharmacologically, by an inhibition of its synthesis using metyrapone, and surgically, by adrenalectomy (ADX).

2. Materials and methods

2.1. Animals

Adult, male Wistar rats (210 ± 10 g) were individually housed in cages (50 cm × 26 cm × 25 cm) in a 12-h light/dark cycle at 22–24 °C, with food and water available *ad libitum*. All experimental procedures were conducted in accordance with the National Institutes of Health's Guide for the care and use of laboratory animals. Additionally, care was taken to minimize the number of animals used in each experiment.

2.2. Exercise paradigm

Each of the exercising rats was given access to a cage that was equipped with 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 that was connected to a separate counter, which was located outside the animal house and monitored the number of revolutions per hour. The number of revolutions for each wheel was recorded every day at 6 am. The sedentary rats were confined to similar cages without access to a wheel. After a 10-day period of exercise, the running wheels were removed from their cages and the rats were trained and tested on the WM task.

2.3. Drugs

The 11beta-hydroxylase inhibitor metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone; Sigma) was injected (S.C.) in a dose of 50 mg/kg in a volume of 2 ml/kg in the dorsal surface of the neck. The drug was dissolved in propylene glycol and diluted with a 0.9% physiological saline solution to reach the appropriate concentration. The final concentration of propylene glycol was 40%. The vehicle control contained the same propylene glycol concentration. This dose was selected on the basis of other studies [30]. All rats received the injections (vehicle or metyrapone) during the 10 nights running period once per day at 6 pm.

2.4. ADX procedure

One week before the initiation of the exercise paradigm, rats were anesthetized by intra-peritoneal injections of 75 mg/kg ketamine and 14 mg/kg xylazine, and underwent either bilateral ADX or corresponding sham surgery. The dorsal midline skin was cut and retracted so that small incisions (1 cm) could be made bilaterally in the abdominal wall near the level of the 1st–3rd lumbar vertebrae. ADX was performed by carefully removing both glands and the surrounding fat with a forceps. The rat's skin was then sutured. Control rats underwent sham surgery, which involved locating the gland in question and lightly touching it with a sterile surgical instrument. Immediately following surgery, all rats were injected with penicillin (0.2 ml, 30,000 IU). The rats were then returned to their home cages and monitored daily. Immediately post-surgery and for the remainder of the study, ADX rats were provided with 0.9% saline in lieu of drinking water in order to maintain their electrolyte balance.

ADX was verified in three ways: (1) successful bilateral removal of undamaged, intact adrenal glands together with the surrounding fat, (2) excessive consumption of drinking solution post-surgery (approximately twice as much as the control rats) and (3) measurements of corticosterone levels in plasma.

2.5. Testing learning and memory using the water maze

A detailed description of the apparatus and the tracking system has been given in previous reports [1,13]. In brief, the water maze was a black circular pool (140 cm in diameter and 60 cm high) that was filled with 22 °C water to a depth of 25 cm.

The WM protocol was a stringent protocol consisting of three trials per day for 5 consecutive days, which has been shown to be a good discriminative test for the effects of exercise on learning and memory [1,13,39]. During each trial, the rat was placed into the water at one of the four cardinal points of the compass (N, E, S, and W), which varied from trial to trial in a quasi random order. The rat had to swim until it climbed on to the escape platform. If the rats failed to locate the platform within 60 s, they were guided by hand to the platform. The rat was allowed to stay on the platform for 20 s during the inter-trial interval. After the last trial, the animal was towel dried and returned to its 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. The rats were allowed to swim for 60 s, during which the latency to reach the platform location, the time spent swimming within a zone, which had a 20 cm radius that was centered either on the original training location (target zone) or on an equivalent location in the opposite quadrant (opposite zone), and the proximity (the average distance from the center of the platform during the probe test) were recorded. The velocity of each animal was also calculated. The analysis of the time spent within a specified radius (zone) and the proximity measure are sensitive measures of the WM probe test performance in terms of detecting group differences [14,25].

Immediately after the probe test, the rats performed a 3-trial visible (cued) platform task in order to assess their motor ability. The platform extended 2 cm above the surface of the water and was moved to a novel quadrant in the pool on each trial, following the same procedure as for the learning and memory test as explained above.

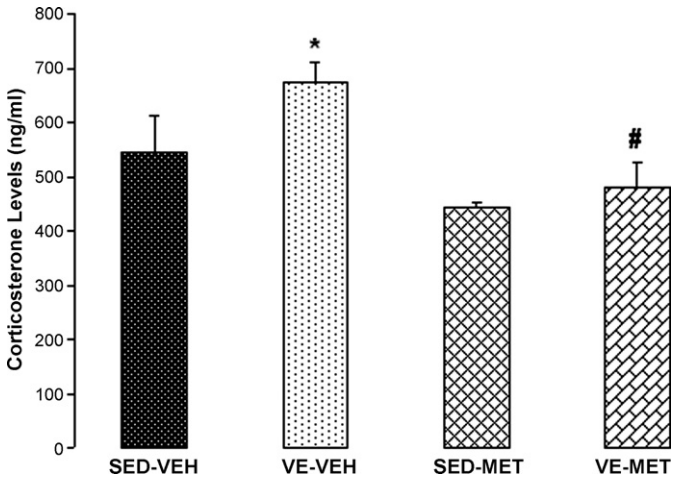

Fig. 1. Effect of blocking of glucocorticoid synthesis by metyrapone on the plasma corticosterone levels in the sedentary and exercise groups. Voluntary exercise increased the plasma levels of corticosterone. Metyrapone treatment decreased the exercise-induced increase in the plasma corticosterone levels. Data are expressed as mean ± SEM of the plasma corticosterone levels. $^{*}P < 0.05$ as compared with the SED-VEH group. $^{\#}P < 0.01$ as compared with the VE-VEH group.

2.6. Radioimmunoassay for corticosterone

To measure corticosterone, the rats were decapitated at the end of the exercise period and their trunk blood was collected in tubes with EDTA, centrifuged (3500 × *g*, 15 min), and the plasma was stored at −70 °C until used for the corticosterone assay. Corticosterone levels were determined by a radioimmunoassay kit (DRG diagnostics, DRG Instruments GmbH, Marburg, Germany). The sensitivity of the assay was 0.39 ng/ml.

2.7. Statistical analysis

Data were analyzed by one-way and two-way analysis of variance (ANOVA) with repeated measures, followed by Tukey's test for multiple comparisons. Student's *t*-test was used to compare two independent groups. Statistical differences were considered significant when $P < 0.05$.

2.8. Experimental protocol

2.8.1. Experiment 1

The aim of this experiment was to examine the effect of metyrapone on the exercise-induced enhancement of learning and memory.

2.8.1.1. Methods. Rats were randomly assigned to 4 groups ($n = 16$ animals per group): sedentary-vehicle (SED-VEH), voluntary exercise-vehicle (VE-VEH), sedentary-metyrapone (SED-MET), and voluntary exercise-metyrapone (VE-MET). The exercising rats were given the 10 days of voluntary exercise according to the procedure described above. All rats received the injections (VEH or MET) once per day (at 6 pm) for a period of 10 days. One day after the end of exercise, half of the animals in each group were decapitated and trunk bloods were collected for the corticosterone assay. The other animals were subjected to learning and memory tests using the WM task.

2.8.1.2. Results. Corticosterone levels: Fig. 1 shows the plasma concentration levels of the sedentary and exercising rats with or without daily treatment of metyrapone. One-way ANOVA revealed significant differences among the groups ($F_{3,28} = 4.83$; $P = 0.007$). Between group comparisons indicated that the plasma corticosterone levels of the VE-VEH group were significantly higher than those of the SED-VEH group ($P < 0.05$). Metyrapone significantly reduced the plasma corticosterone levels of the VE-MET group as compared with those of the VE-VEH group ($P < 0.01$). However, the plasma corticosterone levels of the SED-MET group did not differ significantly from those of their corresponding control (SED-VEH) group. This finding indicates that metyrapone attenuates the exercise-induced increase of corticosterone.