Original Article **Journal of Addiction and Health**
Vol 2, No. 3-4, Summer & Autumn 2010

Received: 26.4.2010
Accepted: 2.8.2010

Does Exercise Deprivation Increase the Tendency Towards Morphine Dependence in Rats?

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Abstract

Background: Exercise deprivation has been concluded to have some negative effects on psychological well-being. This study was conducted to find out whether exercise deprivation may lead to morphine dependence in rats.

Methods: Forty male Wistar rats weighing 162 ± 9 g were housed in clear plastic cages in groups of two under standard laboratory conditions. The study had two phases. In phase I, the animals were randomly divided into exercised (E) and unexercised (UE) groups (n = 20 each) and treadmill running was performed based on a standard protocol for three weeks. At the end of the training period, plasma β-endorphin levels were determined in four rats from each group. In phase II, the animals were provided with two bottles, one containing tap water and the other 25 mg/l morphine sulfate in tap water for a total of 12 weeks. At the end of this phase naloxone was injected intraperitoneally to precipitate morphine withdrawal.

Findings: There was no significant difference between UE and E groups in morphine consumption (mg/kg/wk) [group: $F_{(1,14)} = 0.2$, $P = 0.690$; time: $F_{(11,154)} = 18.72$, $P < 0.001$; interaction: $F_{(11,154)} = 1.27$, $P = 0.245$]. No statistically significant difference between the two groups of animals was seen regarding withdrawal signs.

Conclusion: The study showed that discontinuation of exercise does not increase the tendency of morphine dependence in rats.

Key words: **Exercise dependence, Substance dependence, Oral morphine self-administration, Rat.**

Page count: 7
Tables: 0
Figures: 1
References: 31

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Introduction

It is well documented that exercise not only contributes to both physical and mental health, but also helps people have a better quality of life.[1] Furthermore, physical activity may be beneficial in the treatment of substance dependency.[2,3] On the contrary, the exercise dependence defined as "a multidimensional maladaptive pattern of exercise"[4] has been the subject of much attention and debate in the recent years.[4,5] Several explanations have been proposed for the possible therapeutic effects of physical activity in the treatment of substance dependent patients[3,4,6] and for the pathophysiology of exercise dependence.[4,5] Although the exact pathways and mechanisms have not been clearly established, the β-endorphin theory of endogenous opioids is one of the most popular.[6,7]

Despite the introduction of β-endorphin and its analogues 30 years ago,[8] researchers are still puzzling out a fundamental mechanism for the relationship between endorphins and the "runner's high".[9] "Runner's high" is a feeling of euphoria that exercisers sometimes experience following intense prolonged exercise.[9] Additionally, there is growing evidence that exercise deprivation may lead to a depressed mood.[5,9,10] If endogenous opioid peptides are responsible for both the euphoric state after intense exercise and withdrawal symptoms following exercise deprivation,[9,10] it may be expected that exercise dependent individuals may turn to opiates after a period of exercise deprivation. In an animal experiment opiate drinker rats were randomized into exercise and unexercised groups and it was shown that treatment rats consumed less exogenous opiate during the exercise period than the control group.[11] In another study, Smith and Yancey concluded that chronically exercised rats developed mu-opioid tolerance and physical dependence resembling "those produced by chronic opioid administration".[12]

The present study was carried out to examine whether exercise deprivation increases vulnerability to develop dependence to morphine in rats. In animal experiments, Wistar rats are known to be prone to addiction.[3,13] They are also a suitable model for establishing an excessive treadmill running behavior.[14]

Methods

The experimental procedures employed in this study were reviewed and approved by the Ethics Committee of Kerman Neuroscience Research Center (EC/KNRC/86-42).

Animals and housing

Forty male Wistar rats weighing 162 ± 9 g were obtained from Kerman Neuroscience Research Laboratory. They were housed in clear plastic cages in groups of two under standard laboratory conditions (temperature 20-23 ºC, 12 h light/dark cycle with lights on at 7:00 a.m., 60% relative humidity) with food and water available ad libitum throughout the study.

Exercise schedule

Initially, the animals which refused to run on the treadmill were excluded.[3] Then, the selected rats were randomly divided into exercised (E) and unexercised (UE) groups (n = 20 each). The same person handled the rats throughout the experiment. Treadmill running (Phase I) was performed based on a standard protocol which showed it could significantly raise serum levels of endogenous peptides in exercised rats.[14] The duration of training was three weeks which has been established to be in a range sufficient to elicit change in the endogenous opioid system.[7] Rats were exposed to the running treadmill two times daily, five days a week. Rats were run at the same time in the 10-lane motor treadmill at 5% grade. Each rat had a regular one-minute warming up period at 20 m/min with an incremental increase in treadmill velocity. The highest level of velocity was 40 m/min whose duration was progressively extended from three to six minutes. The final one minute consisted of a warm down at 20 m/min.[14]

Sample collection and radioimmunoassay

At the end of the training period, four rats from each group were randomly selected and anesthetized with CO2 and decapitated. Blood was collected in vacuity tubes containing EDTA and then centrifuged at 4 C for 15 min at 1600 × g. Plasma β-endorphin levels were determined by a rat ß-endorphin RIA kit (Phoenix Pharmaceuticals, Inc., Belmont, CA). All samples were assayed in duplicate. The averages of duplicate tests were used in the data analysis. The intra- and inter-assay coefficients of variation were 4.8% and 7.5%, respectively.

Two bottle choice procedure

During the training period (phase I) rats had continuous access to tap-water by offering two similar bottles and in the testing period (phase II) the animals were provided with the same two bottles, one containing tap-water and the other 25 mg/l morphine sulfate (Darupakhsh, Tehran, Iran) in tap water for a total of 12 weeks.[15] No sucrose was added in the solutions.[15,16] The location of the bottles alternated twice weekly to prevent side preference.[11,15,16] The bottles were weighed on a daily basis for 5 days[15,16] and the results of morphine consumption and water intake were summed up at the end of each week and presented as mg/kg/wk.

Withdrawal signs

Withdrawals were precipitated by intraperitoneal injection of naloxone hydrochloride (2 mg/kg) at the end of phase II. Immediately after naloxone injection the rats were placed individually into a clear container and the withdrawal signs were recorded over a 20-min observation period. Weight loss, however, was assessed 24 hours after administration of naloxone. Withdrawal behaviors were scored according to the method described by Mannelli et al.[17] And only signs that occurred in at least two thirds of animals were analyzed statistically.[17]

Statistical analysis

Each cage was considered as an experimental unit.[18] Continuous variables were presented as means ± SDs. Student's t-test and two-way repeated measures ANOVA were used for data analysis.

Results

There was no significant difference between UE and E groups in morphine consumption (mg/kg/h) [group: $F_{(1,14)}$ = 0.2, P = 0.690; time: $F_{(11,154)}$ = 18.72, P < 0.001; interaction: $F_{(11,154)}$ = 1.27, P = 0.245] (Figure 1). Both groups of rats increased their morphine consumption during the first 4 weeks of phase II similarly, and then reached a plateau (Figure 1).

All rats gained weight across the three-week training period [time: $F_{(3,114)}$ = 360.1, P < 0.001], and no difference was seen in the rate of weight gain between UE (79.8 ± 4.5 g) and E (76.8 ± 4.0 g) rats [$F_{(1,38)}$ = 0.992, P = 0.326; interaction: $F_{(3,114)}$ = 1.3, P = 0.290].

This experiment showed that E rats had a higher mean plasma β-endorphin level than UE rats at the end of phase I (1176.4 ± 64.8 and 716.9 ± 47.8 pg/ml, respectively, P < 0.001).

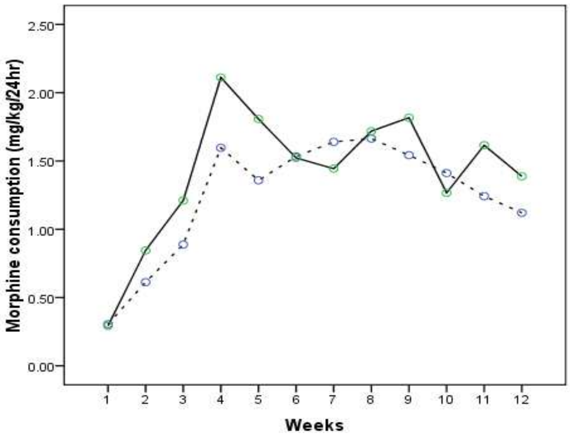

Figure 1. Mean morphine intake (mg/kg/24h) over twelve weeks when rats were provided with two bottles, one containing tap water and the other 25 mg/l morphine sulfate in tap water.

Weight gain [group: $F_{(1,14)}$ = 1.3, P = 0.280; time: $F_{(11,154)}$ = 19.20, P < 0.001; interaction: $F_{(11,154)}$ = 18.12, P <0.001] and water intake [group: $F_{(1,14)}$ = 0.4, P = 0.563; time: $F_{(11,154)}$ = 20.1, P < 0.001; interaction: $F_{(11,154)}$ = 1.1, P = 0.356] did not differ between the two groups over the 12 week two-bottle choice testing (not shown).

The only withdrawal behaviors that were eligible for statistical analysis were shaking and weight loss. Both of these behaviors showed no statistically significant difference between the two groups of animals (total ± SEM count of shaking: 6.5 ± 2.5 and 5.4 ± 1.3, *P* = 0.68 in UE and E rats, respectively; mean ± SEM weight loss: -3.4 ± 1.2 and -4.1 ± 0.9, P = 0.66 in UE and E rats, respectively).

Discussion

In this study, the possible increase in morphine consumption after discontinuation of chronic exercise in rats was investigated. The oral intake of morphine in E rats following exercise deprivation was comparable to that of sedentary animals.

No difference in weight gain was found between UE and E rats during the training period which was congruent with Debruille et al's findings[14] regarding similarity in the exercise protocol. The increase found in the mean plasma β-endorphin level in exercised rats in comparison to sedentary animals is consistent with earlier findings.[11,14] Thus, the findings of this study are in favor of adequate intensity of the exercise protocol.[14,19] The pattern of morphine intake in both groups was similar and exercised deprived rats consumed no more morphine than the control animals on a milligram per kilogram basis (Figure 1). Although the two-bottle choice paradigm is a well established procedure for detecting morphine preference[15,16] and addiction[20], naloxone was administered to both groups on the last day of phase II to evaluate the presence of dependence to morphine. Both tests showed that UE and E rats were comparable with respect to the tendency towards self administration of morphine. These findings are in contrast to other experiments showing a relationship between exercise-induced activation of the endogenous opioid system and self-administration of morphine.[3,11] Some pieces of evidence that support these findings are:

1) The "feel-good effect" is widely regarded as an obvious consequence of regular physical activity, which has been reported to result in a variety of beneficial outcomes, including enhanced mood, anxiolysis and increased pain threshold.[4,5] Furthermore, it is believed that cessation of regular physical activity may lead to withdrawal signs such as depression, anxiety, restlessness and insomnia in humans.[4,5] However, these signs and symptoms, except for anxiety, are not included among the cardinal signs or symptoms of opiate withdrawal, such as GI upset, bone/joint aches, runny nose, yawning and many more.[21] In animal experiments, however, we may see more similarities between these signs and symptoms.[22] If exercise addiction is similar to chronic opioid administration, then exercise deprivation would result in withdrawal signs similar to those of opiate cessation. In order to describe exercise deprivation as an endorphin deficiency state, more experiments need to be done on human volunteers since most athletes do not voluntarily stop exercising.[23]

2) Despite the large amount of experimental data, the exact biological mechanisms of exercise dependence remain unknown.[24] Besides the "opioid theory" of "runner`s high" [22] and exercise dependence[4], several other biological mechanisms including sympathetic arousal and inflammatory cytokines have been implicated in exercise dependence.[24] According to sympathetic arousal hypothesis, exercise dependence is attributed to hormonal changes in catecholamines.[5] This theory is supported by studies on psychological effects of exercise.[5,25] Although hormonal levels of both endogenous opioids[14] and catecholamines change[5] after physical activity, the lack of tendency to morphine consumption in exercised deprived rats requires biological mechanisms other than the endogenous opioid system. It is important to note that in this experiment we examined only β-endorphin levels.

3) According to Cami and Farre, rapid habituation occurs as a person is repeatedly subjected to natural rewards whilst such an adaptive change does not occur following repeated doses of addictive drugs.[26] It is hypothesized that lack of habituation allows addictive drugs to stimulate dopamine release in the nucleus accumbens shell nondecrementally[27] resulting in addictive behavior. Thus, in the case of repeated physical activity, habituation does not allow this process to occur.

4) It is noteworthy to mention that whilst many studies which have attempted to measure self administration of oral morphine in rats use individually caged animals,[15,16] we housed them in groups of two for two reasons; first, it has been documented that isolation per se is conducive to more morphine consumption and may confound the results.[28] Second, since "the occurrence of human addictive behavior usually happens within social environment"[29] we decided to house them in an environment which better imitates the situation of a human drug abuser. Furthermore, no sucrose was added to water due to the activating effect of the endogenous opioid system which may modify withdrawal signs.[30]

5) There is no empirical evidence that discontinuation of exercise is included among the risk factors of drug abuse.[31] An extensive literature review failed to find any such evidence.

In conclusion, this study showed that discontinuation of exercise does not in fact increase vulnerability of rats to morphine dependence.

Conflict of interest: The Authors have no conflict of interest.

Acknowledgement

We thank Dr. Esmaeili Mahani and Dr. Mobasher for their technical advice. This work was the first author's thesis and was supported financially by Kerman Neuroscience Research Center.

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مقاله پژوهشی

مجله اعتیاد و سلامت

سال دوم/شماره ٤-٣/تابستان و پاییز ۱۳۸۹

آیا ترک ورزش سبب افزایش میل وابستگی به مورفین در موش صحرایی می‌شود؟

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تاریخ دریافت: ۸۹/۲/۶

تاریخ پذیرش: ۸۹/۵/۱۱

چکیده

مقدمه: ترک ورزش می‌تواند منجر به عواقب منفی بر سلامت روان افراد شود. این مطالعه با هدف پاسخ به این سؤال انجام شد که آیا ترک ورزش سبب افزایش میل وابستگی به مورفین در موش صحرایی می‌شود؟

روش‌ها: چهل موش صحرایی نژاد ویستار با وزن ۹ ± ۱۶۲ گرم در قفس‌های پلاستیکی شفاف در شرایط استاندارد نگهداری شدند. مطالعه در دو فاز انجام شد؛ در فاز اول، موش‌ها در دو گروه تمرینی (سه هفته تمرین تردمیل) و بدون تمرین قرار گرفتند. سپس از هر گروه، چهار موش مورد اندازه گیری بتا اندورفین قرار گرفت. در فاز دوم، دو بطری آب با و بدون مورفین به موش‌های دو گروه به مدت ۳ هفته داده شد. در پایان تزریق نالوکسان برای موش‌های دو گروه انجام شد.

یافته‌ها: مقدار مصرف مورفین در دو گروه تفاوت معنی‌داری نشان نداد ($P = ۰/۶۹۰$). همچنین نمره علایم ترک مورفین نیز بین دو گروه تفاوت معنی‌داری نشان نداد ($P > ۰/۰۵$).

نتیجه‌گیری: این مطالعه نشان داد که قطع کردن ورزش، تمایل به وابستگی به مورفین را در موش صحرایی افزایش نمی‌دهد.

واژگان کلیدی: **وابستگی به ورزش، وابستگی به مواد، خود مصرفی خوراکی مورفین، موش صحرایی.**

تعداد صفحات: ۷

تعداد جدول‌ها: -

تعداد نمودارها: ۱

تعداد منابع: ۳۱

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