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Voluntary and forced exercises prevent the development of tolerance to analgesic effects of morphine in rats

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ABSTRACT

Objective(s): Morphine is widely used to treat chronic pain. However, its utility is hindered by the development of tolerance to its analgesic effects. Despite the renowned beneficial effects of physical exercise on cognitive functions and signs of morphine withdrawal in morphine-dependent rats, little is known about the roles of voluntary and forced exercises in tolerance to analgesic effect of morphine in rats.

Materials and Methods: In this study, rats were injected with 10 mg/kg of morphine, once daily, SC over a period of 8 days of either voluntary or treadmill exercise. Following these injections, the percent of maximum possible effect (%MPE) of morphine was measured on the 1st, 4th, and 8th days by hot plate test.

Results: Both voluntary and forced exercises significantly increased pain threshold compared to the sedentary group (P<0.05). Voluntary and forced exercises also significantly increased potency of morphine compared to sedentary morphine group (P<0.05). Thus, we concluded that voluntary and forced exercises blocked the development of tolerance during 8 daily simultaneously treatments. When exercising rats were returned to sedentary conditions, sensitivity to the analgesic effects of morphine increased significantly and persisted during sedentary period in the exercising rats. In other words, %MPE of the exercising morphine-group increased significantly compared to saline group (P<0.05).

Conclusion: Our results showed that voluntary and forced exercises may be possible methods for treating the development of tolerance to analgesic effect of morphine in rats.

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Introduction

Although opioids are highly effective for the treatment of pain, their long term use results in complex behavioral changes including tolerance, dependence, and sensitization (1- 2). Morphine tolerance remains a clinical problem because the progressively higher doses of morphine, which are required to relieve the pain, reduce safety and exacerbate morphine dependence (3). It is assumed that changes in synaptic plasticity occurre after long-term morphine use (4). Previouse results have shown that during morphine tolerance-dependence, beta-endorphin and methionine-enkephalin levels decrease in discrete brain regions (5, 6) and plasma (7). Therefore, the reversal or prevention of the synaptic modifications could be a useful method for the treatment of morphine tolerance. In animal studies, both forced and voluntary exercises have stimulated the release of beta-endorphin and other endogenous opioid peptides that are believed to be responsible for increasing the nociceptive threshold (i.e. analgesia) by activating μ-opioid receptors peripherally and centrally, being reported after short- or long-lasting physical activities (8-14). Furthermore, it has been shown that beta-endorphin levels in plasma, pituitary, and whole brain were higher in exercising morphine-treated animals (15). Our previous studies have shown that physical activity through wheel running decreases the severity of naloxone-precipitated morphine withdrawal signs as well as the anxiogenic-like behaviors in both morphine-dependent and withdrawn rats (16, 17).

However, other studies have found that access to a running wheel after chronic exercise reduces the potency and sensitivity to antinociceptive effects of morphine and other mu opioids (18-20), leading to the development of cross-tolerance to exogenously administered opioid agonists (18, 19).

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Given the well-known beneficial effects of physical exercise on behavioral consequences of morphine-dependent and withdrawn rats, and since the analgesic effects of exercise is due to the release of endogenous opioids and significant changes at different levels of the brain, exercise may be a potential method for treating tolerance to morphine in rats. Thus, the aim of the present study was to investigate whether forced and voluntary exercises would prevent tolerance to the analgesic effects of morphine.

Materials and Methods

Animals

Adult male Wistar rats (200–250 g) were used in this study. All rats were individually housed in cages for a 12 hr light/dark cycle at 24± 2°C and had access to food and water *ad libitum*. All procedures were conducted based on the ethical guidelines for the care and use of laboratory animals.

Induction of analgesic tolerance

Morphine sulphate (Temad Company, Iran) was dissolved in physiological saline. Rats were injected once a day for eight days with 10 mg/kg morphine (SC) as described previously (4). Control rats were treated similarly, except that only normal saline was used. All injections were performed in a volume of 0.1 ml/100 g body weight.

Voluntary exercise protocol

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, Novidan Tab, Iran) that was freely rotating against a resistance of 100 g. Each wheel was equipped with a magnetic switch connected to a separate counter, being located outside of the animal house and monitored the revolutions per hour. The number of revolutions for each wheel was recorded every day at 7 a.m. Every rat was required to run a minimum of 100 m each night (16, 17). The sedentary rats were confined to similar cages with no access to a wheel.

This type of exercise, as a short exercise, closely mimics the choices of humans on exercising because animals were dictated regarding the time, speed, and distance of running throughout the experiment (21-22).

Treadmill exercise protocol

Rats in the exercise group were forced to run on a treadmill for 30 min once daily for 8 consecutive days. The exercise load for the exercise group was consisted of running at a speed of 2 m/min for the first 5 min, 5 m/min in the sec 5 min, and then at a speed of 8 m/min for the last 20 min, with 0° inclination as described previously. This type of exercise is a regular mild (a low intensity) treadmill exercise (11). Neither electrical shock nor physical prodding was used to motivate the animals. After every run, the treadmill was cleaned with 70% ethanol solution, wiped and air dried before the next set of four rats were put on the treadmill.

Hot-plate test

Antinociceptive responses were determined at 54°C and defined as animal licking its back paw, as previously described (3). Rats were placed onto a hot-plate maintained at 37°C for 1 min. A cut-off time of 45seconds was imposed to prevent tissue damage. Response latencies are reported as percentage of maximal possible effect (%MPE) [(response latency-baseline response latency)/(cut off latency-baseline response latency)×100].

Statistical analysis

Data are expressed as mean±SEM, and were analyzed using two-way ANOVA, with repeated measures. *Post hoc* analyses included Tukey's test. Statistical differences were considered significant at $P<0.05$.

Experimental protocols

Experiment 1

This experiment examined the effects of voluntary and forced exercises on exercise-induced expression of analgesic tolerance. Rats were randomly assigned to three groups (n=8 rats per group): 1) Sedentary, no injection (Sed); 2) Voluntary exercise for 8 consecutive days without injection (V. Exc); 3) Treadmill exercise for 8 consecutive days without injection (T. Exc). Baseline pain assessment was performed before exercise, and the exercise-induced antinociceptive response was assessed on the 1st, 4th and 8th day of exercise.

Experiment 2

This experiment examined the effects of voluntary and forced exercises on the expression of tolerance to morphine-induced antinociception. In this experiment, rats were randomly assigned to four groups (n=8 rats per group): Saline/Sedentary (Sal/Sed), Morphine/Sedentary (Mor/Sed), Morphine/Voluntary exercise (Mor/V. Exc), and Morphine/Treadmill exercise (Mor/T. Exc). The exercising rats were allowed to exercise during the development of tolerance to morphine, which lasted 8 days. Daily morphine injections in exercising rats were performed after counting the number of revolutions for each wheel or after running on the treadmill. A pain assessment was performed on days 1, 4 and 8 of exercise once before and then 30 min after injection of morphine or saline.

Experiment 3

The purpose of this experiment was to estimate whether there was sensitivity to analgesic effect of morphine in the sedentary period after

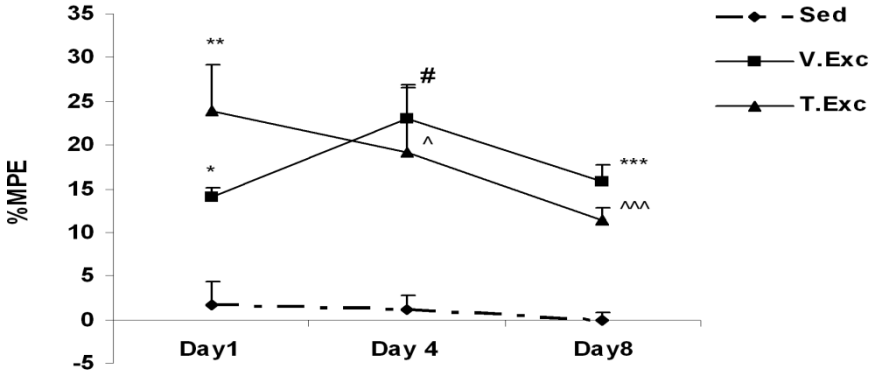

Figure 1. Effects of voluntary and forced exercises on exercise-induced expression of analgesic tolerance. Exercise-induced analgesia followed by running wheel or treadmill over 8 days showed no significant decrease. Data are expressed as mean±SEM. * P=0.049, # P=0.013 and *** P=0.0001 represent the significant difference between the V. Exc and Sed groups on the 1st, 4th and 8th days of exercise, respectively; ** P= 0.001, ^ P=0.041 and ^^^ P=0.0001 represent the significant difference between the T. Exc and Sed groups on the 1st, 4th and 8th days of exercise, respectively (ANOVA, Tukey's test)

disconnection of the 8-day voluntary and forced exercises. In this experiment, the exercising groups were allowed to do voluntary and forced exercises for 8 consecutive days. Afterward, the exercising rats in each group were divided into two groups being injected with either saline or morphine. Saline or morphine was administered daily for 8 days after the end of exercise in sedentary period. In this experiment, rats were randomly assigned to four groups (n=8 rats per group): Voluntary exercise/saline injection in sedentary period (V.Exc/Sal), Voluntary exercise/morphine injection in sedentary period (V.Exc/Mor), Treadmill exercise/saline injection in sedentary period (T.Exc/Sal), and Treadmill exercise/morphine injection in sedentary period (T.Exc/Mor). Exercise-induced antinociception baseline responses were performed on day 8. In order to assess sensitivity to the analgesic effect of morphine in exercising rats, assessment of pain was carried out on days 9, 12 and 16 of the sedentary period, once before and then 30 min after injection of saline or morphine.

Results

Experiment 1

Tolerance to the analgesic effect of exercise following to running wheel or treadmill was not observed in exercising rats.

The average distance run (m) for 8 days of voluntary exercise of the exercising rats was 8977±307. Repeated measures ANOVA revealed significant effects of days ($F_{7, 49}$=2.57, P=0.008). The amount of exercise was markedly increased as exercise days progressed (data are not shown).

The exercising groups exhibited significantly greater response latency (%MPE) over 8 days of running than those of the sedentary control rats (P<0.05). Two-way ANOVA with repeated measures (day) for the response latency of 8 days of voluntary exercise revealed a significant effect of groups ($F_{1, 21}$= 15.39, P=0.0001) and effect of days ($F_{2,42}$=4.07, P=0.028), and no significant interaction between both factors ($F_{2,42}$=2, P=0.112) (Figure 1).

Moreover, two-way ANOVA with repeated measures (day) for the response latency (%MPE) of 8 days of forced exercise with treadmill revealed absence of a significant effect of days ($F_{2,42}$=2.47, P=0.103) and a significant effect of groups ($F_{1,21}$=24.09, P=0.0001), and a significant interaction between both factors ($F_{2,42}$=5.03, P=0.042) (Figure 1). Meanwhile, the response latency during running over 8 days of exercise did not differ significantly between the exercising groups.

Experiment 2

Both voluntary and forced exercises prevented the development of tolerance to morphine antinociception.

The average distance run (m) for 8 days of voluntary exercise in the exercising rats was 9028±323. ANOVA with repeated measures revealed considerable effects of days ($F_{7, 49}$= 6.017, P=0.001) (data are not shown).

Two-way ANOVA with repeated measures (day) for the response latency over 8 days of exercise revealed a significant effect of groups ($F_{3, 28}$=12.52, P=0.0001) and effect of days ($F_{2, 56}$=19.37, P=0.0001), and a significant interaction between both factors ($F_{6, 56}$=8.49, i=0.0001) (Figure 2).

There was a difference in response latencies on the first day of testing between morphine and saline treated groups (P=0.0001), while there were no differences in response latencies on days 4 and 8 of testing between morphine and saline treated groups. In other words, analgesic tolerance in morphine treated rats developed over the eight-day test period. However, forced and voluntary exercises with daily injections of morphine simultaneously attenuated morphine analgesic tolerance compared to sedentary rats receiving morphine ($F_{3, 28}$=12.52, P=0.0001).

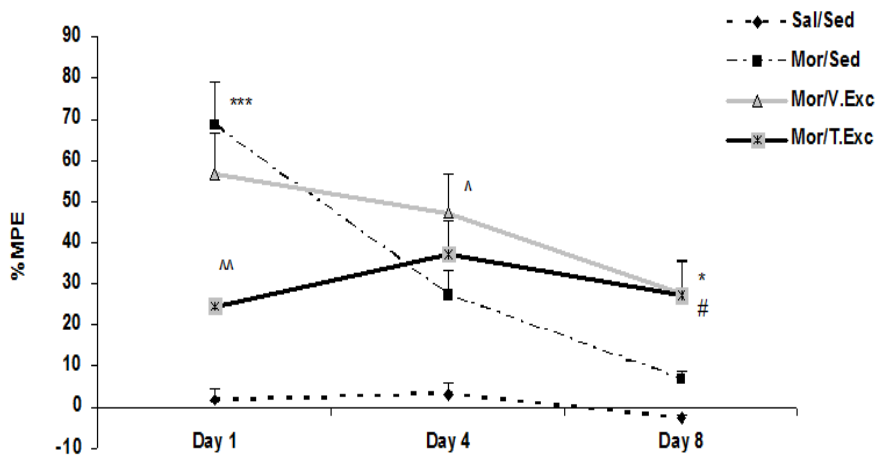

Figure 2. Voluntary and forced exercise can reverse opioid analgesic tolerance compared to sedentary group receiving morphine. Analgesic tolerance in morphine-received rats developed over the eight-day injection period. Voluntary and forced exercise group over 8 days of running significantly increased response latencies compared to sedentary rats receiving morphine

The data are expressed as mean±SEM. *** P=0.0001 represents the significant difference between the Mor/Sed and Sal/Sed groups on the 1st day of injection; ^ P=0.044 and * P=0.017 represent the significant difference between the Mor/V. Exc and the Mor/Sed on the 4th and 8th days of exercise, respectively; ^^ P=0.001 and # P=0.05 represent the significant difference between the Mor/T. Exc and the Mor/Sed on the 1st and 8th days of exercise, respectively (ANOVA, Tukey's test)

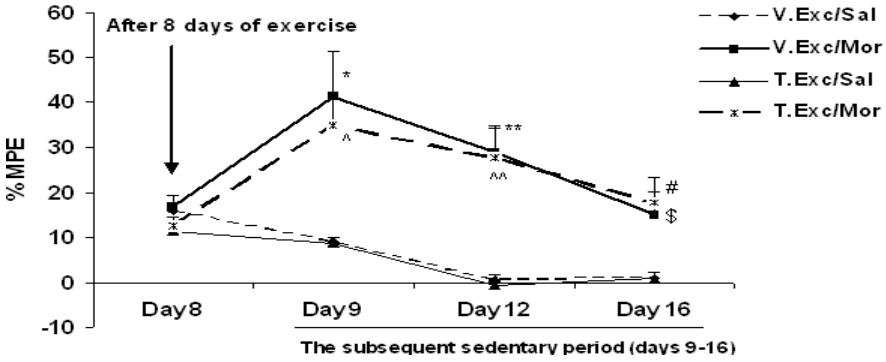

Figure 3. Assessment of morphine-induced sensitization in exercising rats during sedentary period. Exercising rats treated with morphine showed greater antinociception on days 9, 12 and 16 than rats being injected with saline; in other words, sensitivity to the analgesic effects of morphine was persisted at sedentary period in exercising rats, while the analgesic effect of exercise in the control group (receiving saline) terminated 4 days after cessation of exercise. There was no significant difference in the response latency between groups on day 8 of exercise

Data are expressed as mean±SEM. * P=0.015, ** P=0.001 and # P=0.035 represents the significant difference between the V.Exc/Mor and V.Exc/Sal groups; ^ P=0.048, ^^ P=0.002 and $ P=0.05 represent the significant difference between the T.Exc/Mor and the T.Exc/Sal on the 9st, 12th and 16th days of sedentary, respectively (ANOVA, Tukey's test)

Experiment 3

Sensitivity to the analgesic effects of morphine in voluntary and forced exercises groups receiving morphine, persisted during sedentary period.

The average distance run (m) for 8 days of voluntary exercise in the exercising rats (n=16) was 9989±427. ANOVA with repeated measures revealed noticeable effects of days ($F_{7,98}$=7.98, P=0.0001) (data are not shown).

ANOVA with repeated measures (day) for the response latency over 8 days of sedentary revealed significant effect of groups ($F_{3, 28}$ =18.103, P=0.0001) and days ($F_{2,56}$=7.66, P=0.001), but no significant interaction between the two factors ($F_{6,56}$=0.784, P=0.586) (Figure 3). Analgesic effect of exercise in rats receiving saline was prevented 4 days after cessation of exercise, while morphine-induced sensitization persisted during sedentary phase.

Discussion

No study with the same nature has been conducted thus far. This study provides novel evidence that voluntary and forced exercises can preserve morphine analgesic potency that is attenuated by chronic morphine administration.

In this study, eight days of access to running wheels or treadmill was not shown to make tolerance to exercise-induced analgesia.

Akin to previous studies (10, 23, 24), we found that voluntary or forced exercise alters nociceptive threshold. Animal models studies have shown that regular repeated aerobic exercise produces long-

lasting antinociception in untreated animals (19,25-26), as exercise alleviates inflammatory and chronic neuropathic pain (23, 27, 28) and chronic muscle pain (29). The mechanism of exercise-induced long-term analgesia is not fully known. It may be explained in part as activating endogenous opioid-mediated pain modulation systems (12), as most studies have shown that short-term (4 days) (30) or long term (13, 14, 31, 32) repeated exercise increases opioid concentrations of plasma and cerebrospinal fluid. Previous studies have shown that exercise stimulates the release of endogenous opioid peptides approximately 30 min after exercise which remains high for 2 days after the interruption of running (19). One feasible explanation for continues analgesic effects of exercise is possible involvement of nitric oxide (NO) (33), as levels of nitrate in plasma are increased after exercise (34). Overall, these studies have shown that voluntary or forced exercise-induced antinociception remains stable throughout running.

Also, this study showed that the concurrence of voluntary or forced daily exercises with morphine administration delays tolerance to morphine analgesic effects.

This finding seems to be contrast with previous studies stating that during chronic exercise (6 weeks), decreases in sensitivity to the antinociceptive effects of morphine and other mu opioids may reflect the development of cross tolerance between beta-endorphin release during exercise and exogenously injected morphine (18, 19), or reflect a compensatory down-regulation of opioid receptors (35) during exercise. Such discrepancy is probably due to 6 weeks exercise condition prior to analgesic assessment of morphine, while in our study, rats were given free access to 8-day exercise simultaneously with morphine administration.

This is in contrast with earlier studies which demonstrated that after 10 days of forced exercise, pain threshold in exercise morphine-addicted group was decreased compared to non-exercise group. This could be due to the forced running paradigm which is associated with a certain level of stress and the type of treatment (36).

This finding is consistent with our previous results showing that the exercising groups were exposed to voluntary exercise during the development of dependence on morphine (10 days), diminished the severity of the morphine dependency (16). In addition, it may be explained that running reduces the craving for morphine in rats (37), as well as morphine rewarding effects in the conditioned place preference procedure (20). However, these studies did not mention reduction of morphine analgesic. Another possibility is that an exercise-induced increase in BDNF concentrations may lead to increased concentrations of endogenous opioids (12), and it has been shown that BDNF-induced analgesia was reversible by naloxone (38, 39). Furthermore, it has been shown that the increase in BDNF was accompanied by an augmentation in brain serotonergic activity (40). There are probably multiple analgesia systems, including opioid and non-opioid systems following exercise (10).

Thus, the present study showed that free access to voluntary or forced exercise during the development of tolerance to morphine does not decrease sensitivity to antinociceptive effects of morphine over 8 days of running. It also revealed the absence of a significant effect of days on the response latency in exercising rats.

In this study, sensitivity to analgesic effects of morphine remained relatively stable at sedentary conditions in exercising rats.

Our results showed that the analgesic effect of exercise on the exercising rats receiving saline was eliminated 4 days after cessation of exercise, while exercising rats treated with morphine demonstrated greater antinociception on days 9, 12 and 16 than rats injected with saline.

The response latency in exercising rats treated with morphine at day 16 after exercise was %14.9 and %17.9 in voluntary and forced exercise group, respectively, which was much higher than the sedentary rats receiving morphine in Experiment 2 (%6.9). It reflects the stability of sensitivity to analgesic effects of morphine after cessation of exercise training.

This finding has been confirmed by human and animal model studies, which have shown that beta-endorphin levels in the cerebrospinal fluid remained high for approximately 1-2 days after running was terminated (19, 31, 41). Thus, it is believed that higher levels of beta-endorphin increase the nociceptive threshold after training (9, 10). Siuciak *et al* (40) found that increase in serum BDNF levels after exercise may be important for the stability of sensitivities to the analgesic effects of morphine after exercise. Because injecting BDNF to the rat brain produced analgesia 24 hr after injection that reached its maximum levels by day 5 and remained constant for at least an additional 6 days, suggesting no development of tolerance (40).

Conclusion

Either voluntary or forced exercise prevents the development of tolerance to morphine antinociception. Moreover, exercise induces the stability of sensitivity to analgesic effects of morphine. Thus, physical activity may be a possible method for treating the development of tolerance to analgesic effect of morphine in rats.

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Conflict of Interests

Authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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