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ORIGINAL ARTICLE / RESEARCH

Effects of Forced Treadmill Exercise on Pain Threshold in Morphine-Addicted Rats

SARKAKI A R*, SAADIPOUR KH*, BADA VI M*, ALAEI H**, RAHIM F***

ABSTRACT

Background: Animal models comparing rat behaviours are often used in studies characterizing addiction and stress. Aim of this study was evaluation of five or ten days forced treadmill exercise effect on morphine addiction-induced hypoalgesia in young male rats.

Materials and Methods: In this study we used twenty four male Wistar rats weighing 200-300 g. Addicted and non-addicted rats have run as forced exercise on motorized treadmill one hour daily for ten days. Tail-flick latency was tested for each rat three times daily with 10 min intervals at a day before, 5 and 10 days after running on treadmill. A sham group consisted of animals placed on treadmill while its motor was off but electrical shock turned on. Mean of tail-flick latencies was analyzed statistically in sham, ran addicted and non-addicted rats.

Results: The tail-flick latencies were no significant alteration between all groups during 24 hours before forced running (1080 m distances daily). Animals ran 5400 m and 10800 m during 5 and 10 days on treadmill, respectively. Tail-flick latencies showed that pain reflex latency was increased significantly ($p < 0.001$) in E.nA, nE.A, and exercised addicted group (E.A) groups in comparison to nE.nA rats after 5 and 10 days of addiction alone or with forced exercise, but it is significantly reduced in E.A vs. nE.A after 10 days of exercise ($p < 0.001$).

Conclusion: Our data showed that treadmill forced exercise increased pain threshold in non-addicted rats, as well as morphine administration enhanced tail-flick latency in addicted groups after 5 and 10 days of exercise. This finding suggests that elevated stress hormones release followed by forced running and opioid receptor sensitivity associated with morphine administration could be the underlying reason why addicted runners have low pain threshold after 10 days of exercise.

Key words: Morphine, forced exercise, pain, rat

Introduction

Opiates such as morphine are the most effective treatment for pain. Much of what is known about how morphine inhibits nociception has been revealed through laboratory experiments on animals [1]. Furthermore, the studies show that aerobic exercise stimulates the release of β -

endorphin and other endogenous opioid peptides that are believed to be responsible for the increases in nociceptive threshold (i.e. analgesia) after vigorous activity [2-4]. Evidence for the role of opioid receptors in these effects is derived from studies demonstrating that the opioid antagonist naloxone prevents nociceptive

threshold elevation following exercise in normal volunteers [5–8] and attenuates the antinociceptive effects of running in rats with free access to exercise wheels. If exercise is engaged regularly for extended periods of time, sensitivity to the effects of exogenously administered opioids is reduced [9]. Previous studies show that free access to exercise wheels decreases sensitivity to the antinociceptive effects of morphine in the tail-flick procedure in rats [2],[10]. Most studies involving exercise were based on voluntary running paradigms [11], while in the present study we utilized treadmill forced exercise.

In order to evaluate the intensity of clinical pain, patients are often asked to judge the intensity of their pain on a 10 point scale with a score of 10 being the worst pain they ever felt or could imagine and 0 being the score for no pain [12]. In determining the analgesic effects of drugs using the tail-flick procedure, the stimulus intensity is typically adjusted for each animal so that the baseline latencies are approximately equal for all animals. The tail-flick response is believed to involve both spinal and supra-spinal levels of the central nervous system. Two of the most common experimental procedures for evaluation of acute pain and analgesia in lab animals are the “tail-flick” and the “hot plate” techniques. In both of these procedures a fixed nociceptive intensity is used [13–15]. In the present work, we examined the effects of 5 and 10 days treadmill forced exercise on tail-flick latency (as pain threshold) in addicted and non-addicted rats.

Materials and Methods

In this study, we used twenty four male Wistar rats weighing 200–300 g. The study was done i

n Physiology Research Center of Ahwaz Jondishapur University of Medical sciences, Iran. Animals were managed as 5 rats per cage with free access to water and food in the animal houses and in a 12-h light/dark cycle and thermoregulated environment. The animal care and experimental protocol was approved by the Jondishapur academy of sciences ethics committee. Prior to the onset of behavioral testing, all rats were gently handled for 5 days (daily 5 min). Rats were divided randomly into four groups: 1) non-exercise non-addicted group (nE.nA, n=6), which received 1 ml dextrose 5% ip, two times daily during the first 5 days of 10 days exercise and also on 10th day of forced exercise 2) exercise non-addicted group (E.nA, n=6) that received normal saline 3) non-exercise addicted group (nE.A, n=6) which received ip injection of morphine during first 5 days of 10 days exercise (5, 10, 20, 40, 50 mg/kg, two times daily, 8 am and 5 pm) and a single dose 50 mg/kg on 10th day of exercise 4) exercise addicted group (E.A, n=6) that received same doses of morphine and forced exercise on treadmill.

Speed and duration of exercise for two groups (E.nA and E.A) were kept constant at 17-18 m/min, 60 min daily for 10 days. Inclination was varied during 60 min forced exercise. The slope was 0° at first 10 min, 5° at second 10 min, and during next two 20 min periods it was adjusted to 10° and 15°, respectively. The nE.nA and nE.A groups were always placed in a neighboring lane without switching on the treadmill motor for the exact duration as the runners but were not forced to run. Electrical part of treadmill delivered light electric shocks when the rats entered the rear of the test chamber. Both runners and non runners could avoid the shocks by remaining on the treadmill.

Drugs

Morphine sulfate hydrochloride was purchased from Iranian Temad's company. The drug was dissolved in sterile dextrose 5% solution.

Tail-flick test

The thermal intensity of tail-flick was set on degree 35 that corresponds to 50° C temperature. Furthermore, the cut-off time for maximum latencies was set at 10 sec to avoid tail tissue damage. The location of tail-flick thermal stimulus was at 8 cm from tip of the tail. The baseline latencies were determined for all groups

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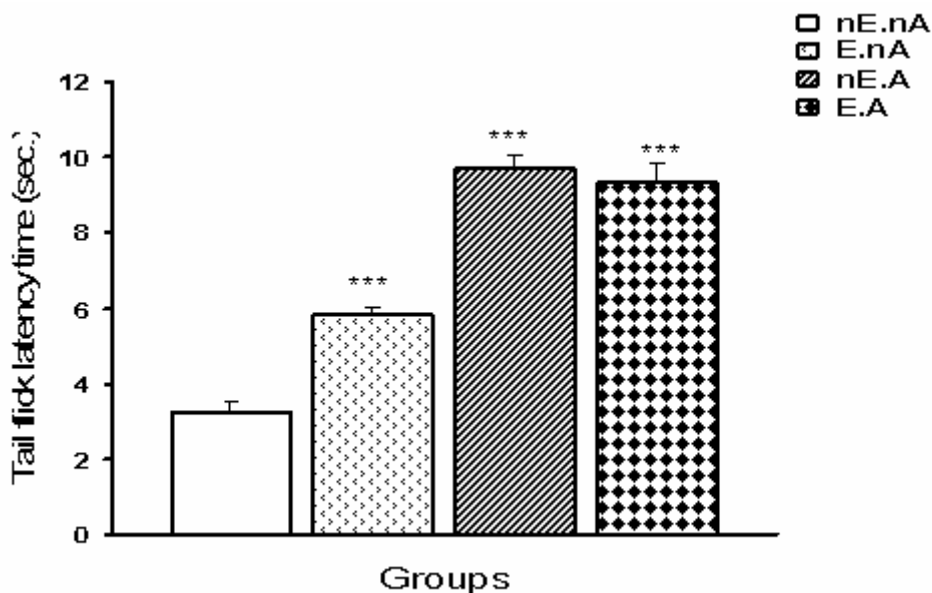
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at a day before the rats involved with addiction and exercise.

Statistical analysis

All data collected were analyzed with using the statistical software (SPSS, Ver.13). Latencies (baseline, 5th and 10th day of exercise) were

analyzed by one-way ANOVA and Tukey's post-hoc test. Paired T-test was used for comparison of latencies between days for each group. The score reported was mean \pm SEM of two trials before, after 5 and 10 days exercise. The p-value less than 0.05 considered as significant.



[Table/Fig 1] Tail-flick latency (Mean \pm SEM) after 5 days of exercise and addiction in all groups. ***P<0.001 Vs nE.nA (ANOVA, Tukey's test, n=6).

Results

Effect of exercise and addiction on pain threshold

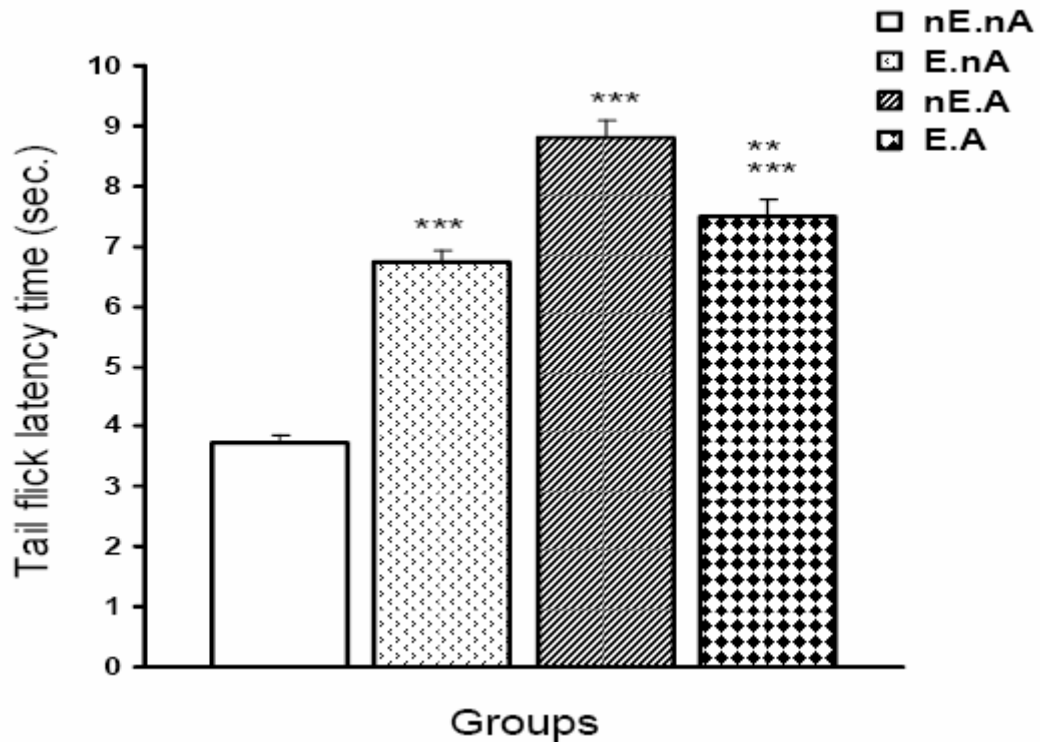
Twenty four hours before rats have run on treadmill as forced exercise and not exposed to morphine, the baseline tail-flick latencies showed no significant alteration between all groups. Tail-flick latency after 5 days ([Table/Fig 1]) and 10 days ([Table/Fig 2]) of forced exercise and addiction showed that pain threshold was enhanced significantly ($p<0.001$) in E.nA, E.A and nE.A groups in comparison to nE.nA group ([Table/Fig 1]). After 10 days of forced exercise, pain threshold in E.A group was decreased significantly ($p<0.001$) as compared to nE.A group ([Table/Fig 2]).

Alteration in pain threshold after 5 or 10 days of exercise and addiction

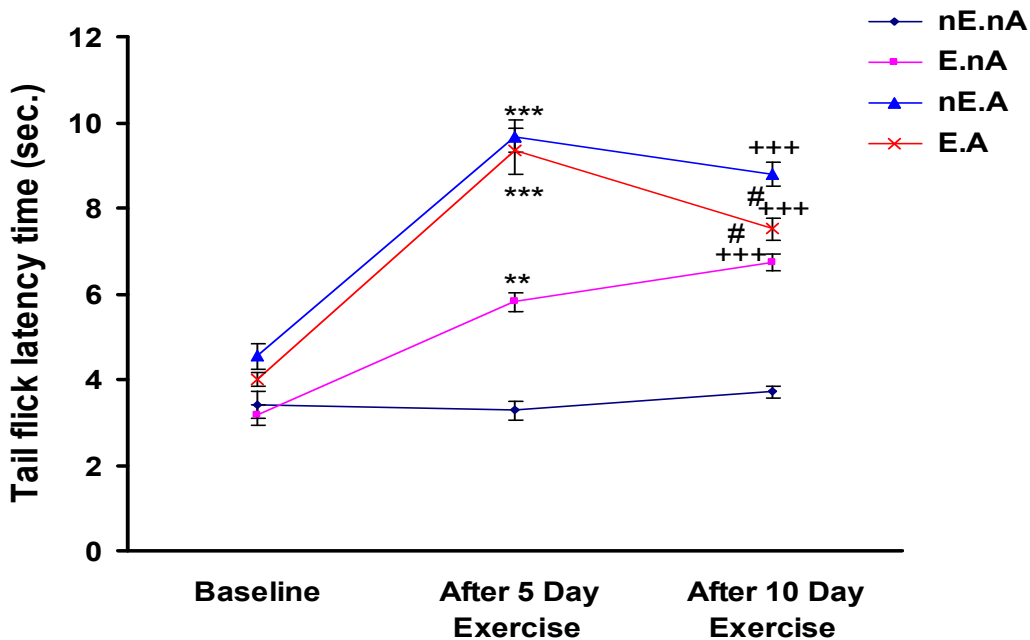
Pain threshold enhanced significantly ($p<0.001$) after 5 and 10 days exercise as compared to baseline of experiment time for all groups except nE.nA group. Also comparison between 5 and 10 days, showed significant decrease in nE.A and E.A groups ($p<0.05$) respectively, but it was increased significantly ($p<0.05$) in E.nA group ([Table/Fig 3]).

Discussion

This study shows that treadmill forced exercise increases pain threshold in non-addicted rats. The findings in the present work appear to be relevant to clinical analgesia because the relative potency of morphine and other analgesic compounds is consistent between acute nociceptive tests in animals and clinical application. In the present study, morphine administration enhanced tail-flick latency in addicted group after 5 and 10 days of exercise.



[Table/Fig 2] Tail-flick latency (Mean ± SEM) after 10 days of exercise and addiction in all groups. ***P<0.001 Vs nE.nA, **P<0.01 Vs nE.A (ANOVA, Tukey's test, n=6).



[Table/Fig 3] Alteration of pain threshold (Mean ± SEM) between days in each groups (*comparison between baseline and 5th day, +comparison between baseline and 10th day and #comparison between 5th and 10th day of forced exercise (Paired- samples t- test, n=6, ***P<0.001, **P<0.01, +++P<0.001, #P<0.05).

The exercise couldn't alter pain threshold in E.A group after 5 days. We found variability in group's baseline tail-flick latencies that might be attributed to the individual differences and also unlikely breeding between rats groups.

We report that 10 days of treadmill forced exercise significantly decreased pain threshold in addicted rat. This is consistent with earlier studies, which demonstrated that aerobic exercise stimulates the release of β -endorphin and other endogenous opioid peptides that are believed to be responsible for the increases in nociceptive threshold (i.e. analgesia) reported after vigorous activity [2-4]. Evidence for the role of opioid receptors in these effects is derived from studies demonstrating that the opioid antagonist naloxone prevents elevation in nociceptive threshold following exercise in normal volunteers and attenuates the antinociceptive effect of running in rats with free access to exercise wheels [9],[16],[17]. In rats, for example, free access to exercise wheels decreases sensitivity to the antinociceptive effect of morphine in the tail-flick procedure [18]. Previous investigators have proposed that this decrease in sensitivity may reflect the development of cross tolerance between β -endorphin released during exercise and exogenously administered morphine [19-21]. Furthermore, it is known that free access to exercise wheels had fewer β -endorphin binding sites than sedentary rats, an effect that was presumed to reflect a compensatory down-regulation of opioid receptors during exercise [22],[23]. Such reduction in the numbers of opioid receptors such as kappa [24] and mu have also been reported during chronic treatment with exogenous opiates. Additionally, it has been suggested that increased activation within the dynorphin pathway contributes to hyperalgesia in morphine tolerant animals as well as neuropathic pain and chronic pain states in humans and laboratory animals [25-29].

It has been cited that long-term voluntary wheel exercise could reduce pain opioid receptors sensitivity but our findings show that after 10 days forced treadmill exercise had same effect. This could be due to the forced running paradigm, which is associated with a certain level of stress [30] mechanistically; which in turn might be attributed to the release of adrenal stress hormones, epinephrine, cortisol, corticosterone in the rats, as a consequence of

the emotional arousal [31]. The elevated stress hormones followed by forced running and opioid receptors sensitivity associated with morphine administration could be the underlying reason why addicted runners had low pain threshold.

As a conclusion, our study showed that treadmill forced exercise increased pain threshold in non-addicted rats. Morphine administration also enhanced tail-flick latency in addicted groups after 5 and 10 days exercise after 5 days exercise comparing to the baseline, but forced exercise decreased the pain threshold in addicted group after 10 days comparing to the 5 days of exercise.

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