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Original Article

The Effect of Harmane on Hyperalgesia Induced by Stressed Male Mice in the Presence and Absence of Moderated Exercise

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Abstract

Introduction: Physical exercise has positive effects on stress-induced pain response, while chronic stress persuades a negative effect on cognitive functions. Depending on the nature, duration and intensity of the stressor, it can repress pain (stress-induced analgesia) or exacerbate pain (stress-induced hyperalgesia). Furthermore, beneficial effects of Harmane on stress processes have been reported in rodents. This study aimed to investigate the effects of Harmane and moderate physical activity (associated or not) on pain response in restraint stressed mice.

Materials and Methods: Harmane was injected intraperitoneally at doses of 0.1, 0.3 and 0.6 mg/kg, every other day until 28 days, and pain response of the adult NMRI mice was detected using the hot-plate test

Results: The results exhibited that Harmane, at all doses used, did not alter pain perception in mice; however, 3- but not 6 and 9-day restraint stress (3 hours per day) induced hyperalgesia per se. In addition, Harmane reduced hyperalgesia in 3-day stressed mice, while moderate treadmill running (10 m/min for 30 min/day, 5 day/week) caused hyperalgesia in 6- and 9-day stressed mice. Furthermore, the hyperalgesia induced by moderate treadmill running in 9-day stressed mice restored by Harmane.

Conclusion: The findings indicated that Harmane has a protective effect on hyperalgesia induced by stress per se or potentiated effect of moderate treadmill running in stressed mice.

Keywords: Harmane, Restraint stress, Treadmill running, Pain, Mice

1. Introduction

Stress is an exciting state in which a person experiences an unusual limitation and reveals emotional, physical and cognitive responses [2]. Although technology and mechanization in the world are increased, stress is extending for a variety of social and economic reasons and many people suffer from it. In general, there are two types of stress: malignant and benign. The malignant stress is harmful, unpleasant and pathogenic, but the benign stress is beneficial and pleasant, which gives a positive emotion to the person [3]. The effects of stress are related to the severity and duration of the stimuli; for example, acute stress stimulates individual's thinking and makes him aware of the environment; hence, it promotes success

and prosperity. On the other hand, chronic stress leads to weakening the immune system and reducing body defense against diseases [4]. Stress also leads to different responses to pain. It has been reported that stress reduces pain in people; when a person is exposed to stress, the pain is decreased [5]. Regarding this issue, Ford and colleagues (2008) showed that stress induces many hormonal and neurological changes that lead to analgesia [6, 7]. On the other hand, it has also been reported that stress increases pain: when a person is exposed to stress, the pain is increased [8]. The effect of stress on pain response depends on the severity, duration and type of stress [8]. Dubai and colleagues showed that, stress increases pain response in mice [9].

The role of physical activity in preventing diseases has been recurrently reported; for example, previous research has shown that physical activity, especially aerobic exercise, is useful for health and proper brain function, especially in stress-related diseases [10]. Physical activity prevents many diseases, such as hypertension, heart disease, overweight and osteoporosis, and has psychological benefits, including reducing anxiety and stress [11]. Furthermore, some results have indicated that moderate exercise decreases depression, anxiety, obsession and psychosis [12, 13].

β-carbolines (also called harmala) can be divided into three structural groups including, fully unsaturated pyridine ring (harmane), the dihydro derivative (harmaline) and the tetrahydroderivatives depending upon their degree of ring saturation [14, 15] and have been found in common plant-derived foodstuffs, plant-derived beverages and plant-derived inhaled substances (tobacco) [16]. Harmane is an opioid-dependence indicator that reduces depression and stress in humans and rodents [17]. It has been revealed that Harmane inhibits monoamine oxidase (MAO) in the brain [18]. Furthermore, it can increase the metabolism of dimethyl tryptamines, dopamine and catecholamines in the brain. Moreover, Harmane has been identified as an anti-depressant [1] and has neuroprotective effects [19, 20]. Some studies indicated that harmane as endogenous ligand has very high selectivity for Imidazoline receptors [21, 22]. Activation and deactivation of imidazoline receptors induced diversity of responses including cell proliferation, regulation of body fat, neuroprotection, inflammation, depression and pain perception [21].

Due to the mentioned points, the present study aimed to evaluate the effect of ineffective dose of Harmane, alone or in combination with moderate treadmill running, on pain responses concurrent due to the stress in male mice.

2. Materials and Methods

2.1 Animals

The male NMRI mice (24-28 grams) from a breeding colony at the Institute for Cognitive Science Studies, Tehran, Iran were used in this study. Standard conditions (a 12-h light/dark cycle with lights on from 07:00–19:00 and controlled temperature 22 ± 2°C) was provided. Discrete cages were also used to keep the mice (8 per cage). A week before the beginning of the experiments, the mice were freely fed and got acquainted with the experimenter and environment. Ethics Committee of the Faculty of Science of the University of Tehran which corresponds to the national guidelines for animal care and use approved all procedures and methods used in this research.

2.2. Restraint Sress Model

In chronic restraint stress experiments, mice were restrained in Plexiglas mesh restrainers, which prevented forward or backward movement, 3 hours/day for 3, 6 and 9 days [23]. Mice were returned to their own cages after restrain treatment.

2.3. Moderated Treadmill Running

Moderated treadmill exercise was carried out 10 m/min during 30 minutes per day for 5 days per week during 4 weeks. All mice pre-exercised to habituate to treadmill running (Borj sanat, Iran) 10 m/min during 20 minutes per day for 5 days. Sedentary mice were placed on the treadmill that was turned off for 30 min once a day.

2.4. Hot-plate Apparatus

Hot plate apparatus is a sheet getting hot by electric current (BorjSanatAzma Co, Tehran, Iran). In this study, hot plate sheet was cleaned with 70% ethanol and then, all the rats were placed on the plate. The start time (zero) was determined and as soon as the rats started to lick their paws or change their steps, their basic tolerance was recorded. The temperature of hot plate apparatus was set at 50±1 centigrade degree. The reaction time to thermal pain was recorded and licking paws or special changes in rats' steps considered as a response to thermal pain. Cut-off time of the test was 1 minute [24, 25].

2.5. Drugs

The drugs used in the present study was Harmane (1-methyl-9H-pyrido [3,4-b] indole, C12H10N2) from Sigma (St. Louis, MO). The time of injections and doses of compounds used in the experiments were chosen according to published works in scientific literature [23, 26]. Harmane was dissolved in sterile 0.9% NaCl solution and the compound was stirred for 1 h before obtaining the final solution (0.1, 0.3 and 0.6 mg/kg) and was injected peritoneally.

2.6. Experimental Design

Each group consisted of eight mice that were used only once. The experiment was finished in 28 days and at the 29th day the hot-plate test was done.

2.6.1. Experiment 1

This experiment assessed the impact of Harmane administration on pain response concurrent due to the stress in male mice. Four groups of non-exercised animals (sedentary treadmill mice) received various doses of Harmane (0, 0.1, 0.3 and 0.6 mg/kg), once/48h during 28 days (from 8th day to 28th day). Hot-plate test was carried out one day after the last administration of Harmane.

2.6.2. Experiment 2

In experiment 2, mice were separated into four sets of four groups as follows:

Restraint stressed groups: to investigate the effect of different periods of restraint stress on pain response, four groups of non-exercised mice were subjected to 0, 3, 6 or 9 consecutive days of restraint stress with the duration of 3 h/day. Hot-plate test was performed one day after stress exposure period.

Harmane combined with restraint stress: to investigate the effect of ineffective dose of Harmane on pain response, four groups of non-exercised mice received Harmane at the dose of 0.6 mg/kg and then, were subjected to restraint stress in various days with the same stress protocol. Then, hot-plate test was done at 29th day.

Treadmill running combined with restraint stress: to investigate the effect of moderate physical exercise on pain response, four groups of mice were placed in treadmill and were subjected to restraint stress in various days with the same stress protocol. Then, hot-plate test was done at 29th day.

Harmane in combination with treadmill running with restraint stress: to investigate the effect of ineffective dose of Harmane and treadmill running on pain response, four groups of mice received Harmane and then, were placed in treadmill and were subjected to restraint stress in various days within last days. Then, hot-plate test was done at 29th day.

2.7. Statistical Analysis

For statistical analyses, SPSS was used (SPSS for Windows, version 19.0). By using one- or two-way ANOVA and Post

hoc Tukey test, the significance difference between groups were assessed. All values are reported as mean ± standard error (SE). Statistical significance was considered as $p<0.05$.

3. Results

3.1. Effect of Harmane on Pain Response in Male Mice

Fig. 1 shows that, doses of 0.1, 0.3 and 0.6 mg/kg of Harmane did not alter the hot-plate latency. In fact, Harmane did not induce hyperalgesia [F (3, 28) =0.837, P =0.485; Fig. 1]. In conclusion, Harmane at all doses did not alter pain perception.

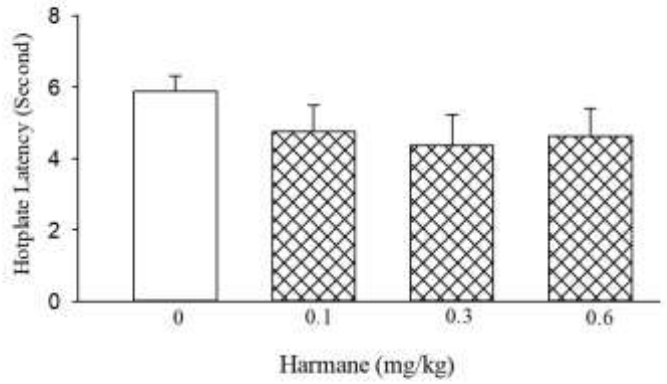

Fig.1

Figure 1. The effect of Harmane at the doses of 0, 0.1, 0.3 and 0.6 mg/kg on pain perception in male mice.

3.2. Effects of Stress, Harmane and Exercise on Pain Response in Male Mice

Fig.2 (Panel 1) showed that, restraint stress for 3 days decreased hot-plate latency, while 6 and 9 days' restraint stress did not alter hot-plate latency, [F (3, 28) =13.406, P<0.001]. In conclusion, only 3 days' restraint stress induced hyperalgesia.

Two-way ANOVA and post-hoc analysis showed that, the ineffective dose of Harmane (0.6 mg/kg) reversed the effect of restraint stress on hot-plate latency in mice treated with 3-day restraint stress (stress effect: [F (3, 56) =13.406, P<0.001], Harmane effect [F(1, 56) = 11.065, P<0.001] and Harmane-stress interaction effect [F (3, 56) =3.449, P<0.03; Fig.2, Panel 2].

Similar analysis indicated that, treadmill running decreased the hot-plate latency in mice treated with 6- and 9-day restraint stress. [stress effect: F (3, 56) = 13.406, P<0.001; treadmill running effect: F (1, 56) = 29,647, P<0.001; stress-treadmill running interaction: F (3, 56) = 24.969, P<0.001; Fig.2, Panel 3]. In conclusion, moderate treadmill running induced hyperalgesia in mice treated with 6- and 9-day restraint stress.

Fig. 2 (panel 4) indicated that Harmane, at the dose of 0.6 mg/kg reversed the effect of treadmill exercise on the hot-plate latency in mice treated with 9-day restraint stress [stress effect: F (3, 56) = 4.406; P<0.01; combination of Harmane and treadmill running effect: F (1, 56) = 111.897, P<0.001; stress-combination of Harmane and treadmill running interaction: F (3, 56) = 5.988, P<0.01]. In fact, Harmane, when used with exercise, can reduce hyperalgesia caused by exercise in 9 days stressed mice.

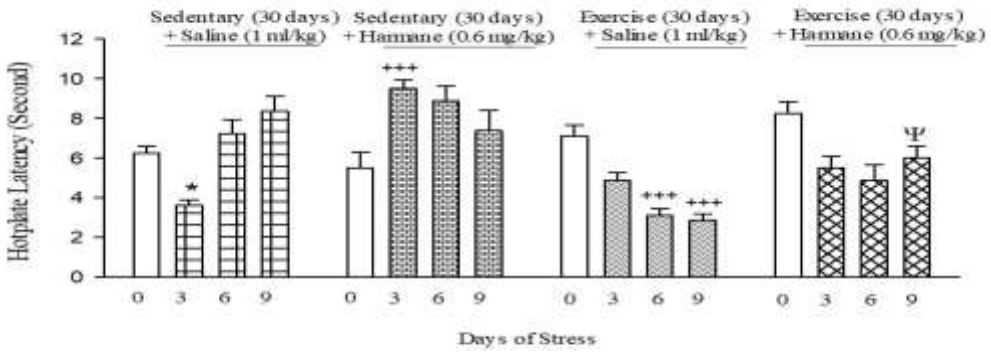

Fig.2

Figure 2. The effect of stress (panel 1), Harmane (panel 2), exercise (panel 3) and exercise plus Harmane on pain response in male mice (panel 4). * P<0.05 compared to control group in the panel 1. +++ P <0.001 compared to respective control groups in the panel 1. ψP <0.05 compared to respective control groups in the panel 3.

4. Discussion

The purpose of this study was to investigate the effect of Harmane and moderate exercise on pain response concurrent due to the stress in male mice. The main finding of the present study was that 3 but not 6 and 9 days' stress could induce hyperalgesia. There are several studies, indicating that acute or chronic stress increased nociceptive response to pain via decrease of pain threshold, resulting in hypersensitivity [27, 28], followed by repeated social defeat [29], repeated forced swim stress [30] and repeated restraint stress [31]. Compelling studies postulated that that brain-derived neurotrophic factor (BDNF)-trkB-KCC2-GABA pathway induced a critical role in hyperalgesia induced by inflammation and BDNF and KCC2 have been demonstrated to be involved in stress [32, 33].

The results of the present study also indicated that chronic administration of Harmane (at applied doses in this study) decreased hyperalgesia in mice treated with 3-day stress. It has been demonstrated that Harmane inhibits amino oxidase (MAOI), which facilitates the metabolism of DMT (dimethyl tryptamine) and other tryptamines and increases the level of dopamine and catecholamines in the brain [1]. This property can explain the effect of Harmane on cognitive processing. Substantial evidence implicates that chronic restraint stress reduces hippocampal neurogenesis and leads to neuron destruction, which induces hippocampus-dependent cognitive deficit [18]. On the other hand, Harmane increases proliferation of cells which differentiate and mature into neurons and prevents the destruction of neurons in the brain [19, 20]. It is likely that chronic pretreatment with Harmane via increase in hippocampal neurogenesis and MAO inhibitors (monoamine oxidase) prevents the impairment effect of restraint stress. Also, Ghazaleh et al. indicated that Harmane increases release of serotonin in brain via possible role of serotonergic system and the beneficial effect of Harmane as a combination of antidepressants or anxiety-provoking [34].

Along with Harmane, treadmill exercise induced hyperalgesia in mice treated with 6- and 9- day stress. Previous studies have reported that, in forced exercise model (running on a treadmill by electric shock), the axis of the adrenal pituitary hypothalamus (HPA) is activated and can increase the level of adrenal steroids and increases stress in mice, while continuous running is not a stressor [35]. In this study,

moderate treadmill running produced hyperalgesia in stressed mice. Regarding these findings, this study examined whether an ineffective dose of Harmane can potentiate the effect of treadmill running on pain response in stressed mice, or not. The results showed that Harmane reversed hyperalgesia induced by concurrent treatment of treadmill running on 9- but not 3 and 6 days stressed mice. It is concluded that, Harmane can be used as a therapeutic option for stress-induced hyperalgesia and in moderate intensity exercise.

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Conflict of interest
The authors declare no conflict of interest.

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