Physiology & Behavior 129 (2014) 214–220

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Role of nitric oxide in altered nociception and memory following chronic stress

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HIGHLIGHTS

- Chronic swim stress induces hyperalgesia and impairs avoidance learning.
- Chronic swim stress alters locomotion through NO pathway.
- Pretreatment with NOS inhibitors protects against stress-induced alterations.

ARTICLE INFO

Article history:
Received 9 October 2013
Received in revised form 5 February 2014
Accepted 28 February 2014
Available online 11 March 2014

Keywords:
Stress-induced hyperalgesia
Nitric oxide
Passive avoidance learning

ABSTRACT

Introduction: Chronic stress alters sensory and cognitive function of mankind and animals. Sub-chronic swim stress is known to induce a prolonged hyperalgesia which is mediated through the NMDA and opioid systems. Nitric oxide is a soluble gas which acts as a retrograde messenger that modulates the release of the mentioned neurotransmitters. It is also involved in nociception and memory. The objective of the current study was to evaluate the role of NO pathway in nociception and memory impairments induced by sub-chronic swim stress.
Methods and materials: A three session forced swimming stress protocol was administered to the rats. Pretreatment with L-NAME (10 mg/kg, i.p.), L-Arginine (10 mg/kg, i.p.) or saline was made before each swimming session. Anxiety-like behavior, nociception and passive avoidance (PA) learning were evaluated 24 h after the last swim stress session.
Results: Swim stress altered locomotion and anxiety-like behaviors in the open field test. Reduced thermal threshold was observed in the nociceptive measurement after swim stress. Pretreatment with L-NAME could reverse the reduced threshold. A decreased step through latency was observed in the PA paradigm after swim stress, which could be inhibited by pretreatment with L-NAME.
Conclusion: The results of this study indicate that sub-chronic swim stress impairs nociception and PA learning. NO pathway seems to have a modulatory role in these alterations. Further studies are suggested to examine the protective effect of NOS inhibitors on stress-induced impairments in sensory and cognitive function.

1. Introduction

Acute stress leads to analgesia (stress-induced analgesia: SIA) [1], while under certain circumstances, stress exacerbates pain, a situation which is called stress-induced hyperalgesia (SIH) [2,3]. Although SIA physiology is extensively studied, many aspects of SIH are yet unclear, and different transmitters are suggested to be involved in its physiopathology [4,5].

It is previously shown that stressors can alter amygdala dependent fear conditioning and passive avoidance (PA) learning and interestingly, different stressors have different effects on PA learning [6–10].

Chronic swim stress has been introduced by Quintero et al. [3] as an animal model of SIH and previous studies have shown that opioids and NMDA receptors mediate hyperalgesia following chronic swim stress [3,11].

Nitric oxide (NO) is a membrane soluble neurotransmitter which is implicated in the formation of memory and synaptic plasticity and acts by modulating the release of other neurotransmitters such as GABA and glutamate [12]. Several studies have shown that eNOS (epithelial nitric oxide synthase) and nNOS (neural nitric oxide synthase) are abundantly found in brain areas responsible for anxiety responses and memory formation including hippocampus and amygdala

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http://dx.doi.org/10.1016/j.physbeh.2014.02.054
0031-9384/

[13–16]. It is previously shown that NO has a modulatory effect on learning and memory in passive and active avoidance learning [17–20]; additionally, the involvement of spinal NO pathway in mediating nociception and hyperalgesia following inflammation [21] and neuropathic injury [22] has been shown. Furthermore, the role of NO pathway in NMDA induced hyperalgesia in the rat formalin test has also been shown [23].

L-Arginine as a precursor of NO and L-NAME (L-NG-nitroarginine methyl ester) as inhibitor of NOS were chosen for the pharmacologic manipulation of NO pathway as previously used in other studies [24,25].

Based on previous findings implicating a role for NO pathway in nociception and the impact of chronic stress on learning and memory, we first tested to see whether any changes occur at the passive avoidance learning following chronic swim stress, then in the second part, we evaluated the effect of NO pathway activation and NO synthesis inhibition prior to each swimming session on hyperalgesia and altered PA learning in the sub-chronic swim stress.

2. Method and materials

2.1. Animals and the procedure

All the procedures were performed in accordance with the regulations set by the Kerman University of Medical Sciences ethics committee regarding experiments on vertebrate animals [ethics committee code: EC/KNRC/92-7]. Maximum effort was made throughout the experiments to minimize discomfort of the animals. Adult male Sprague Dawley rats (180–250 g) were used in this study. Animals were housed individually in a room with controlled temperature with a 12/12 h light/dark cycle and had free access to food and water. All the procedures were performed between 7:00 and 16:00 h by two experienced persons.

Our study consists of 12 experimental groups (each consisting at least 8 rats): 1. Forced swim (FS) and nociception (hot-plate, tail flick, OFT tests); 2. FS and PA tasks (PA task, open field test (OFT)); 3. sham swim (SS) and nociception; and 4. SS and PA tasks and the same grouping pre-treated with saline, L-NAME (10 mg/kg, i.p.) or L-Arginine (10 mg/kg, i.p.) 30 min before each swimming session. Our pilot studies revealed that administration of the dosages of L-NAME and L-Arginine used in this study did not affect any of the parameters evaluated as compared to the control group; therefore, we did not include the sham + drugs in the analysis section.

Baseline measurements were made for rats in the nociception group at day 0; post-stress tests were performed in day 4 after swimming stress. Nociception was measured in days 4, 8 and 12 to confirm the prolonged hyperalgesia observed in previous studies [3].

To assess the effect of pretreatment with L-NAME and L-Arginine, measurements were made 24 h after the last swimming session and data were compared among all study groups.

2.2. Drugs

L-NAME (Sigma, USA) and L-Arginine (Sigma, USA) were purchased from a local provider. Pre-treatment with either L-NAME or L-Arginine (both 10 mg/kg, i.p.) was performed 30 min prior to each swimming session. Dosage of both drugs was chosen based on a pilot study and previous studies [26,27].

2.3. Sub-chronic swim stress

Rats were brought to the laboratory 1 h before applying stress to them. The FS group was subjected to swim stress by placing them in a plastic cylinder (30 cm diameter, height 50 cm) for 10 min in 24–26 °C water on day 1. On days 2 and 3, the same swim stress was given to the animals for 20 min. Sham rats were subjected to sham swim by placing them in a cylinder containing only 2–4 cm of water [3]. Rats were allowed to dry in a cool environment and efforts were made to minimize handling of the animals to avoid possible novelty stresses which might affect the results. After 3 consecutive days of swim stress or sham swim, we evaluated the PA task in the days 4 and 5 and measured the nociceptive responsiveness in the days 4, 8 and 12. Open-field test [28] was used to evaluate the mobility and anxiety-like behavior of rats. It was performed on the day 4 at least 2 h before the other procedures.

2.4. Open-field test

Animals were brought to the testing environment 24 h after the last swimming session and after a 1 h acclimation period, animals were placed in the middle of an open field and the horizontal and vertical activities of the rats were recorded for a period of 5 min and then analyzed using Ethovision software [version 7.1], a video tracking system for automation of behavioral experiments [Noldus Information Technology, the Netherlands]. The apparatus consisted of a square arena [56 × 56 × 20 [H] cm] made of black wood and its floor was divided by lines into 16 squares dividing the field into central and peripheral squares. The following behavioral parameters were recorded for each rat: total distance moved [TDM, cm]; time spent in center and periphery and frequency of grooming and rearing [as a measure of vertical activity]. At the end of each session, rats were removed from the open field and the experimental chamber was cleaned with a damp cloth and dried [28].

2.5. Hot-plate and tail-flick

Nociception was assayed 24 h after the last swimming session and 2 h after OFT. Hot plate test was used to measure reaction time to the thermal stimuli [29]. In this procedure, pain sensitivity was evaluated by using a device (LE710 model, Lsi LETICA, Spain) that contained a plate with a diameter of 19 cm and a Plexiglas wall with a height of 30 cm. Plate temperature was adjusted to 52 ± 0.5 °C. Reaction time to thermal pain was considered as the time between the onset of test and the beginning of nociceptive response including whether licking hind-paw or jumping (maximum cut off was considered 45 s to avoid tissue damage) [28].

Tail flick test was used to evaluate the nociceptive response to acute thermal noxious stimuli at spinal level. This measure was used due to the fact that response to heat noxious stimuli to the tail of the animal is a reflex [29], and we evaluated to see whether reflex movements are affected by swim stress or not. The animals were restrained in a restrainer cage with their tail hanging free and allowed to adapt for 30 min before testing. The lower 5 cm portion of the tail was marked. This part of the tail was put under the burning light and the response time to the pain was considered as the time between turning the burning light on and tail drawn out.

2.6. Passive avoidance (PA) learning

PA learning was assessed using inhibitory passive avoidance paradigm. A shuttle-box device with dimensions of 100 × 25 × 25 which consisted two parts was used. First, the animal was placed in the light arena of the shuttle-box apparatus then after 5 s, the door was opened and the animal was allowed to go to the dark sector, then the door was closed without electric shock and the animal was placed in the cage. This step was repeated again 30 min later and the third time that the animal entered dark sector, an electric shock was administered to the animal (0.5 mA, 1.5 s).

24 h after training, the retention test was performed to evaluate passive avoidance memory and learning; in this step the animal was placed in the light arena of the shuttle-box apparatus. Animals were placed in the light section. After 30 s, the door was opened and the time before the first entry of the animal to the dark section was considered as step-through latency (STL) during a 300 s interval. If the animal did not enter the dark sector, STL was considered as 300 s and the