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Forced treadmill exercise can induce stress and increase neuronal damage in a mouse model of global cerebral ischemia

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ABSTRACT

Physical exercise is known to be a beneficial factor by increasing the cellular stress tolerance. In ischemic stroke, physical exercise is suggested to both limit the brain injury and facilitate behavioral recovery. In this study we investigated the effect of physical exercise on brain damage following global cerebral ischemia in mice. We aimed to study the effects of 4.5 weeks of forced treadmill running prior to ischemia on neuronal damage, neuroinflammation and its effect on general stress by measuring corticosterone in feces. We subjected C57bl/6 mice (n = 63) to either treadmill running or a sedentary program prior to induction of global ischemia. Anxious, depressive, and cognitive behaviors were analyzed. Stress levels were analyzed using a corticosterone ELISA. Inflammatory and neurological outcomes were analyzed using immunohistochemistry, multiplex electrochemoluminescence ELISA and Western blot. To our surprise, we found that forced treadmill running induced a stress response, with increased anxiety in the Open Field test and increased levels of corticosterone. In accordance, mice subjected to forced exercise prior to ischemia developed larger neuronal damage in the hippocampus and showed higher cytokine levels in the brain and blood compared to non-exercised mice. The extent of neuronal damage correlated with increased corticosterone levels. To compare forced treadmill with voluntary wheel running, we used a different set of mice that exercised freely on running wheels. These mice did not show any anxiety or increased corticosterone levels. Altogether, our results indicate that exercise pre-conditioning may not be beneficial if the animals are forced to run as it can induce a detrimental stress response.

1. Introduction

Physical exercise is regarded as a promising treatment and complement to pharmacological treatments in several different neurological diseases (Svensson et al., 2014). Exercise can affect both the adrenergic and corticosteroid systems involved in stress response as well as the microglia function and other cells and signaling molecules involved in inflammatory processes (Svensson et al., 2014).

In the central nervous system (CNS), microglial cells are the major source of pro-inflammatory cytokines (Kim and de Vellis, 2005; Carson et al., 2006). In response to neuronal injury, such as stroke, resident microglia are immediately activated and start to proliferate and produce pro-inflammatory cytokines (Banati et al., 1993; Barone et al., 1997). It has been shown in both patients and animal models that cerebral ischemia leads to an inflammatory response, systemically as well as in the brain (Lambertsen et al., 2012). Neuroinflammatory response can further aggravate the neuronal damage and administration of the pro-inflammatory cytokine IL-1β after induction of cerebral ischemia in animal models can exacerbate the brain injury (Yamasaki et al., 1995). Microglial cells or their inflammatory mediator molecules may therefore be suitable targets for treating or preventing deleterious neuroinflammation following brain ischemia (Yenari et al., 2010). Several studies have shown that the levels of pro-inflammatory

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cytokines decrease, and the levels of anti-inflammatory cytokines increase following physical exercise (Mota et al., 2012; Piao et al., 2013). Indeed, treadmill running in animals has been shown to reduce the microgliosis and inhibit the release of pro-inflammatory cytokines, as well as reducing lesion size and cell death in the hippocampus and prevent short-term memory disturbances following cerebral ischemia (Austin et al., 2014; Lovatel et al., 2014; Sim et al, 2004, 2005).

Out-of-hospital cardiac arrest is a severe complication that can lead to global cerebral ischemia with pronounced neuronal damage. The survival rate is very low, below 10%, and patients that survive are often affected by severe neurological injury and life-long cognitive and motor disabilities (Sasson et al., 2010). In an experimental setting, global cerebral ischemia can be modeled in mice by transient occlusion of the common carotid arteries (Olsson et al., 2003). Global cerebral ischemia results in neuronal cell death in vulnerable brain regions such as hippocampus (Kirino and Sano, 1984; Back et al., 2004) evolving during the first week after the ischemic insult (Bottiger et al., 1998). By altering detrimental neuroinflammatory reactions, physical exercises could make the brain more resistant to ischemic injuries.

On the negative side, exercise can also induce a chronic stress response, which may result in detrimental effects in the event of a brain injury. For example, forced running exercise in rodents can lead to anxiety and increase the levels of the stress hormone corticosterone in serum (Leasure and Jones, 2008; Brown et al., 2007). It has been shown that stress can evoke a pro-inflammatory response in the brain, with increased expression of NLRP3 inflammasome involved in the cleavage and secretion of pro-inflammatory IL-1β (Gadek-Michalska et al., 2013; Frank et al., 2014). Therefore, the overall positive effect of physical exercise in experimental models could potentially be masked by the stress response.

To the best of our knowledge, the effects of treadmill exercise pre-conditioning on stress and neuroinflammatory responses following global cerebral ischemia have not previously been studied in mice. Therefore, the aim of this study was to investigate the effect of pre-conditioning forced treadmill running on stress response, neuroinflammation, neuronal damage and behavioral alterations following global cerebral ischemia. An additional aim was to compare the stress response after forced running with the response after voluntary running.

2. Material and methods

2.1. Animals

All proceedings and animal treatment were in accordance with the guidelines and requirements of the government committee on animal experimentation at Lund University. We used 63 male C57Bl/6 mice, aged 8–10 weeks, weighing 22–27 g that were obtained from Charles River. The mice were housed in standard laboratory cages (3–7 animals per cage), with sawdust bedding and free access to water and food. They were allowed to acclimatize for at least 5 days before testing. The holding room had a 12:12 h light-dark cycle. The mice were weighed at the day of exercise introduction, and thereafter once every second week until the induction of global cerebral ischemia. Thereafter the mice were weighed 3–4 days as well as 10–12 days after ischemia. When the experiment was initiated, the mice were assigned to different groups ensuring an even distribution in body weight and age.

2.2. Experimental outline

An overview of the experimental outline can be seen in Fig. 1. Briefly, some of the animals were subjected to treadmill running exercise for 4.5 weeks. During the third week of exercise their anxious and motor behavior was assessed once with an Open Field test. After 4.5 weeks of exercise, some of the animals were subjected to ischemia. Behavioral tests were then conducted 5–15 days after ischemia, after which, the animals were sacrificed and samples were collected (15–16 days after ischemia).

2.3. Running exercise

2.3.1. Treadmill running exercise

Originally, the mice were divided into two different treatment groups, one subjected to exercise and one that was not subjected to any exercise (sedentary). Exercise consisted of 30 min treadmill running at a speed of 25 cm/s with no inclination of the treadmill (5-lane treadmill, Harvard Apparatus, Panlab). The mice were exercised 3 days/week for 4.5 weeks of which the first week was an introduction week with a lower speed and duration of the exercise. On the first day of the introduction, the mice were subjected to 10 min of walking/running at a speed increasing from 5 to 18 cm/s. The second day of the introduction consisted of 20 min of running with speeds up to 25 cm/s. During the third and last day of the introduction, the duration was increased to 30 min, with a speed of 25 cm/s. To motivate the mice to run the researcher pushed them with a small stick if needed. If the mouse refused to run it could be motivated by a transient and light electric stimulation from the grid at the beginning of the treadmill platform. After this, if the mouse persisted in its refusal to run, it was removed from the treadmill and re-introduced to it at a later time point. In this study, all mice without exceptions had to be motivated at least some times by pushing them with the small stick at each day of exercise. Four of our best running mice even had to be motivated with a light electric stimulation at up to three different days. Despite this, several mice refused to run after repeated trials. These mice were excluded from the exercise procedure, and referred to as “bad runners”. Exercise took place in a room separated from the housing room to which all mice were transferred and kept during the exercise procedures in order to minimize environmental confounders among the mice not subjected to the exercise protocol.

2.3.2. Voluntary wheel running

To investigate the effect on stress response resulting from the enforcement to run compared to voluntary running exercise, our main study was complemented with mice subjected to voluntary wheel running. For this, 10 male C57Bl/6 mice at an age of 12 month were used. These mice had the same housing conditions as the mice in the main study, except that they were single caged. Six of these

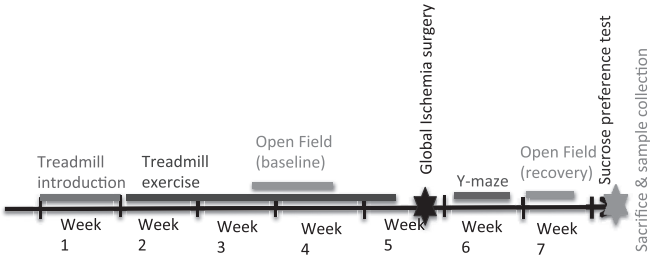

Fig. 1. Experimental Design. Mice in the exercise groups were introduced to treadmill running for a week and thereafter subjected to 3.5 weeks of exercise. During this time an Open Field test was conducted. After 3.5 weeks of exercise some of the exercised and non-exercised mice were subjected to global brain ischemia. The mice were allowed to recover for 3–4 days before Y-maze test was conducted. Then, a new Open Field test was performed followed by a sucrose preference test the night before the mice were sacrificed.