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Research report

Central immune overactivation in the presence of reduced plasma corticosterone contributes to swim stress-induced hyperalgesia

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ABSTRACT

Although it is widely known that immunological, hormonal and nociceptive mechanisms are altered by exposure to repeated stress, the interplaying roles of each function in the development of post-stress hyperalgesia are not completely clear. Thus, we wanted to establish how interleukin 1-beta (IL-1β), corticosterone and microglia interact to contribute in the development of hyperalgesia following repeated forced swim. Rats were subjected to either forced swim, sham swim or non-conditioned. Each group was then treated with minocycline, ketoconazole, or saline. Thermal nociception was measured via the hot plate test, before and after the behavioral conditioning, whereas blood and lumbar spinal cord tissue samples were obtained at the end of the protocol. Serum levels of corticosterone, spinal tissue concentration of IL-1β and spinal OX-42 labeling (microglial marker) were determined. Rats exposed to forced swim stress developed thermal hyperalgesia along with elevated spinal tissue IL-1β, increased OX-42 labeling and relatively diminished serum corticosterone. Pre-treatment with minocycline and ketoconazole prevented the development of thermal hyperalgesia and the increase in IL-1β, without significantly modifying serum corticosterone. These results suggest that the development of forced swim-induced thermal hyperalgesia requires the simultaneous presence of increased spinal IL-1β, microglial activation, and relatively decreased serum corticosterone.

1. Introduction

The stress response can lead to either analgesia or hyperalgesia, depending on the duration and intensity of the stimulus. Acute stress is most commonly associated with the appearance of analgesia (Butler and Finn, 2009) whereas chronic stress has been more frequently linked with the onset of hyperalgesia (Quintero et al., 2000; Wang et al., 2013). Previous studies have reported that repeated forced-swim stress produces a long term increase in the behavioral response to noxious stimulation in rats, alongside increased neuronal activity in nociceptive areas of the spinal cord (Quintero et al., 2003). Similarly, persistent pain states are observed in patients with anxiety, depression, fibromyalgia, and chronic fatigue syndrome after exposure to maintained periods of stress (Dersh et al., 2002).

Central neuroimmunological mechanisms have been suggested as key elements in the development of chronic pain phenomena. Models of chronic pain in rodents, including nerve injury-induced neuropathic pain and chronic inflammatory pain, have demonstrated the involvement of microglia in the facilitation of nociceptive transmission through the production of pro-inflammatory cytokines, especially IL-1β, capable of acting at different pain modulation areas in the central nervous system (Poole et al., 1995; McMahon et al., 2005; Zhang and Huang, 2006; Watkins et al., 2007a,b; Eliav et al., 2009). Studies in animal models also show that exposure to chronic stress is connected with microglial activation, increased release of pro-inflammatory cytokines (including TNFα, IL-6 and IL-1β) and visceral hyperalgesia (Raison and Miller, 2003; Bradesi et al., 2009; Voorhees et al., 2013; Capoccia et al., 2013). In clinical studies, patients with post-traumatic stress disorder (PTSD) spontaneously display higher levels of pro-inflammatory cytokines in peripheral blood mononuclear cells (TNFα, IL6 and IL-1β) and also following LPS-challenges (Gola et al., 2013).

It is known that CNS immunity phenomena, including microglial activity as well as synthesis and release of cytokines, are significantly affected by the hypothalamus-pituitary-adrenal (HPA) axis (Webster et al., 2002). On the other hand, changes in circulating levels of glucocorticoids are known to occur as part of the stress response (Reeder et al., 2004; Reagan et al., 2008). In addition, decreased levels of circulating corticosterone have been found in: (a) a rat model of PTSD concomitant with the development of

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mechanical allodynia and thermal hyperalgesia (Zhang et al., 2012), and (b) a mouse model of chronic psychosocial stress along with visceral hiperalgesia (Tramullas et al., 2012). This evidence highlights that changes in both the HPA axis activity and the immune function are involved in chronic stress-induced hyperalgesia (Blackburn-Munro and Blackburn-Munro, 2001; Fries et al., 2005; Chang et al., 2009; Sominsky et al., 2013). Yet, it is not completely clear the way the HPA axis, immune and nociceptive functions are connected as to lead to the development of enduring cutaneous pain after repeated exposure to stress. Thus, the aim of this study was to elucidate how the interplay between IL-1β, corticosterone and microglia contribute to the development of the hyperalgesia following repeated forced-swim stress.

2. Methods

2.1. Animals

Male Sprague-Dawley rats, weighing 150–300 g, (Vivarium, Faculty of Veterinary Sciences, University of Zulia, Venezuela) were individually caged in the same room where behavioral studies took place. The rats had food (rat chow, Ratarina®, Protinal, Valencia, Venezuela) and water ad libitum. They were divided according to type of behavioral conditioning and pharmacological treatment. For behavioral conditioning, animals were divided into three groups: forced-swim, sham-swim, and non-conditioned. A subset of animals from each behavioral group was pretreated with either ketoconazole or minocycline and the rest with normal saline (control vehicle). All the animals were subjected to the hot-plate test to estimate thermal nociceptive threshold. At the end of the behavioral protocol, animals were anesthetized with ketamine and xylazine. In subsets of each experimental group, blood samples were then intracardially collected and the lumbar segment of the spinal cord was surgically extracted after intracardiac perfusion with cold phosphate-buffered saline solution (PBS), for measurement of serum corticosterone and spinal tissue IL-1β, respectively. Finally, microglial activation in the lumbar spinal cord was determined by immunohistochemistry in saline-pretreated non-conditioned, sham-swim and forced-swim animals, and in minocycline-pretreated forced-swim animals.

2.2. Behavioral protocol

2.2.1. Forced-swim

Rats were placed inside a plexiglass cylinder (30 cm diameter; 50 cm height), filled with a volume of water sufficient to ensure the complete submersion of the body of the animal with the exception of the head (30 cm height); water temperature oscillated between 24 and 26 °C. Animals were subjected to three consecutive conditioning sessions: a 10 min session on the first day, followed by a 20 min session on the second and third day of the protocol.

2.2.2. Sham-swim

Rats were placed inside a plexiglass cylinder with a volume of water barely sufficient to ensure that the four paws were submerged and in direct contact with the bottom of the cylinder (water level ~2–3 cm) while the rest of the body was above the surface of the liquid, with no need for the rat to swim. Rats were subjected to three consecutive conditioning sessions: a 10 min session on the first day, followed by a 20 min session on the second and third day of the protocol.

2.2.3. Non-conditioning

Rats were not exposed to any behavioral conditioning protocol (never placed inside the plexiglass cylinder), remaining mostly undisturbed in their cages except for the injection of saline, ketoconazole or minocycline.

2.3. Pharmacological treatment

2.3.1. Minocycline

A subset of sham-swim and forced-swim rats received a 40 mg/kg dose of minocycline one hour before each conditioning session. Non-conditioned rats received the drug at the same time of the injection of the other two groups. Minocycline was dissolved in normal saline and administered i.p. (Zhang et al., 2006)

2.3.2. Ketoconazole

A subset of sham-swim and forced-swim rats received a 25 mg/kg dose of ketoconazole one hour before each conditioning session. Non-conditioned rats received the drug at the same time of the injection of the other two groups. Ketoconazole, forming a complex with tartaric acid and cyclodextrin, was dissolved in normal saline and administered i.p. (Mantsch and Goeders, 1999; Redenti et al., 1999)

2.3.3. Vehicle (normal saline)

A subset of sham-swim and forced-swim rats received a 100 μl dose of normal saline one hour before each conditioning session. Non-conditioned rats received the vehicle at the same time of the injection of the other two groups.

2.4. Thermal nociception: hot plate test

Thermal nociception was assessed via the hot-plate test. The rat was placed on a hot plate (model 39D, IITC, USA) with a surface temperature of 52.5 °C. The thermal nociceptive threshold was determined by the latency response, i.e., the time in seconds elapsed from the placement of the rat on the plate until it showed a pain-related behavioral response (licking/biting of hind paws or jumping). The animal was placed on the hot plate for a period of time no longer than 45 s in order to avoid tissue damage. Two measurements were made for each animal: before the first conditioning session (basal thermal nociceptive threshold) and 24 h after the last conditioning session (post-stress nociceptive threshold). In every case, the observer was unaware of the conditioning protocol and pharmacological treatment of each animal.

2.5. Determination of IL-1β in spinal cord tissue homogenates

2.5.1. Tissue extracts

A subset of animals from each group was used to determine spinal lumbar tissue concentration of IL-1β. Forty-eight hours after the last conditioning session, rats were subjected to deep anesthesia and intracardiac perfusion with phosphate buffered saline (PBS: 0.1 M, pH 7.4) and then the lumbar segment of the spinal cord was surgically dissected and collected. Spinal lumbar segments were homogenized in 300 μl of Tris Buffer (50 mM, pH 7.4), which contained 1 mM EDTA, 100 mM NaCl, 1 μg/ml aprotinin-isopropanol and 100 μg/ml phenylmethylsulfonyl fluoride (PMSF) (Sigma Chemical Co., USA). Each sample was then transferred to a 1 ml tube and centrifuged at 7000 × *g* for 20 min, at a temperature of 4 °C. Supernatant was collected and analyzed for antigenic detection of IL-1β.

2.5.2. Measurement of tissue IL-1β

Concentration of IL-1β was determined in the lumbar spinal tissue, using a commercially available sandwich-ELISA kit and following manufacturer specifications (R&D Systems, Inc, USA).