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Exercise preconditioning reduces neonatal incision surgery-induced enhanced hyperalgesia via inhibition of P38 mitogen-activated protein kinase and IL-1β, TNF-α release

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ABSTRACT

Neonatal surgery leads to enhanced hyperalgesia to noxious stimulation in adulthood via a mechanism caused by enhanced phosphorylated (p)-p38 expression in microglia. We tested the effect of exercise on reducing enhanced hypersensitivity primed by neonatal incision surgery. Adult female Wistar rats, with or without neonatal incision surgery at postnatal day (P) 3, received right hind paw plantar incision surgery under anesthesia at P44. The rats performed wheel-running exercise from P22 to P41. Paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) were measured and ipsilateral spinal cords were collected for protein quantification. For PWT and PWL, exercise reduced the pain index after incision surgery at P44 in rats with neonatal surgery ($P < 0.01$). Western blots showed that exercise suppressed P-p38 expression relative to adult rats without neonatal surgery ($P < 0.05$). Results of ELISA showed that exercise reduced IL-1β and TNF-α ($P < 0.05$) concentration in the ipsilateral spinal cord. Exercise preconditioning is an effective approach to reducing enhanced adult hyperalgesia primed by neonatal surgery. The mechanism may be explained by exercise-induced inhibition of P-p38 activation and IL-1β, TNF-α release.

1. Introduction

The neonate survival rate has greatly increased in the past several decades, and a host of neonates require intensive care unit admission and invasive interventions. Neonates in the intensive care unit undergo numerous procedures, most of which are heel pricks for blood sampling and endotracheal suction (Barker and Rutter, 1995). Several studies have reviewed the long-term effects of untreated neonatal pain (Hermann et al., 2006a,b; Schwaller and Fitzgerald, 2014; Walker et al., 2009), and found that pain in neonates can cause a long lasting change in the vulnerable developing nervous system. In adulthood, neonatal surgery rats present hyposensitivity to non-noxious stimulation and enhanced hypersensitivity to noxious stimulation compared with rats without a neonatal pain experience (Walker et al., 2009). Similar to animal models, long-term alteration of pain sensitivity to neonatal surgery still exists in school-aged children (Hermann et al., 2006a,b; Hohmeister et al., 2009). The enhanced pain hypersensitivity to noxious stimulation is presented by increased pain amplitude and duration. This lead to an increased analgesic requirement, prolonged analgesia duration in the perioperative period and longer hospital stay (Peters et al., 2005).

Research has found that neonatal surgery primes spinal cord microglia, which contributes to long-term pain circuit plasticity (Beggs et al., 2012b), Pharmacological inhibition of phosphorylated (p)-p38 expression—which occurs exclusively in microglia in the early phase after surgery—completely reversed the enhanced hyperalgesia (Schwaller et al., 2015; Soens et al., 2015). P-p38 activation in microglia generates a multitude of inflammatory mediators, including IL-1β, IL-6 and TNF-α, which lead to pain hypersensitivity (Baldassare et al., 1999; Ji et al., 2009; Xing et al., 2011). Few effective strategies for treating the pain hypersensitivity are known. Considering that enhanced pain perception is detrimental to adults, and that parents are unwilling to give neonates analgesic drugs, it is critical to explore new methods to prevent this lasting change.

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Exercise preconditioning is a non- pharmacological method of reducing inflammation. Studies have demonstrated that exercise preconditioning inhibits microglia activation (Sung et al., 2012; Yoo et al., 2015), as well as inflammatory response to traumatic brain injury (Mota et al., 2012) and sciatic nerve crush injury that generate neuropathic pain (Bobinski et al., 2011; Kuphal et al., 2007). Thus, exercise preconditioning may reduce the enhanced hyperalgesia primed by neonatal surgery. This research was designed to test the effect of exercise preconditioning on P-p38 expression, release of inflammatory mediators, and pain behaviors in adult surgery rats primed by neonatal surgery. Results found that exercise preconditioning reduced the enhanced hyperalgesia, P-p38 expression and IL-1β, TNF-α release primed by neonatal surgery.

2. Materials and methods

2.1. Animals

The experiments were performed in accordance with the Animal Care and use Committee of Shanghai Jiao Tong University and animal care guidelines of the National Institutes of Health. Pregnant female Wistar rats were obtained from the Shanghai Experimental Animal Institute and were kept in a temperature- and humidity-controlled environment on a 12-h light/dark cycle (lights on between 7:00 AM. to 19:00 P.M.) with *ad libitum* access to food and water.

2.2. Group allocation

After gestation, rat pups were sexed and only females were included in the study. The litters were weaned at postnatal day (P) 21. Rats were allocated to four groups across litters in case of potential litter variability. The groups were: IN, neonate anesthesia + adult anesthesia surgery; EX + IN, neonate anesthesia + exercise + adult anesthesia surgery; nIN + IN, neonate anesthesia surgery + adult anesthesia surgery; nIN + EX + IN, neonate anesthesia surgery + exercise + adult anesthesia surgery. Behavioral testing was performed on 32 rat pups (8 in each group). Spinal cord collection and assay were performed on 40 rat pups (10 in each group), including Western blot and ELISA on 6 rats and immunohistochemistry on 4 rats. Neonatal surgeries were performed at P3, and adult surgeries were performed at P44. Anesthesia time and maternal separation in neonatal IN and EX + IN groups were roughly similar to those of the rats in the surgery groups. The surgery was performed by one researcher, and the behavior test was performed by another researcher blinded to the group allocation.

2.3. Plantar incision surgery

Plantar incision surgery was conducted under inhalational anesthesia with isoflurance (4% for induction followed by 1.5–2.5% for maintenance) using an anesthesiameter (Ugo Basile Gas Anesthesia System). The surgery followed the procedures used in previous studies (Beggs et al., 2012b; Walker et al., 2015). At P3, the pup's right hind plantar was incised along the middle line from the midpoint of the heel to the level of the first footpad. After the skin incision, the plantaris muscle was elevated and longitudinally incised. Then the skin was sutured with 4–0 silk and the pup was immediately returned to its litter under maternal care. The suture was removed 5 days after the surgery. At P44, all the rats received the same incision surgery as at P3. The incision was closed and the rats returned to their cage.

2.4. Exercise protocol

Rats weaned at P21 and exercised from P22 to P41. Exercise was provided 6 days per week. After 5 min acclimation, the wheel was started at a low speed of 5 m/min for 5 min to warm up, and then the wheel velocity slowly increased to reach and maintain a constant speed. For the first 3 days, the rats received 10 min running at a speed of 5 m/min. Running at a speed of 8 m/min was provided for the remainder of the first week, then increased to 10 m/min in the second week and 15 m/min in the third week. The rats' performance was rated according to previous study (Dishman et al., 1988): 1 = refused to run, 2 = below average runner (sporadic, stop and go, wrong direction), 3 = average runner, 4 = above average runner (consistent runner, occasionally fell back on the treadmill), 5 = good runner (consistently stayed at the front of the treadmill). The rats rated 3 or higher in the first 3 days were included in the study. An electrical control unit (YLS, Yiyan scientific) with a negative electric stimulus, set at 0.2 mA, was used to run the exercise sessions.

2.5. Paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) test (Schwaller et al., 2015)

PWT and PWL were measured at P22, 29, 36, 43 (2 days after exercise) to evaluate the effects of exercise. After the last mechanical test at P43, rats received incisional surgery at P44. Behavior measurements were then made at P45, 47, 49, 51, 54, 59 for 8 rats in each group. PWL was tested after the PWT behavior test.

2.5.1. PWT test

For the mechanical paw withdrawal threshold test, the rat was placed on a metal grid in a Plexiglass Box 50 cm above the table. After 15 min of acclimation, the mechanical withdrawal threshold was measured using Electronic Von Frey Hair (IITC Life Science Inc, Woodland Hill, CA, USA). We used a slow-increasing force to stimulate the adjacent site of incision; the threshold was recorded if a sudden paw withdrawal was observed. This was repeated 3 times in 5 min intervals. The average threshold was calculated and used for analysis.

2.5.2. PWL test

To measure thermal paw withdrawal latency, the rat was placed in the Plexiglass box on an elevated glass table. After 15 min of acclimation, a beam of radiation emitted by a Plantar Test AnalgesiaMeter (IITC Life Science Inc., CA, USA) was used to stimulate the adjacent site of the injured hind paw. 30 s was set as the cutoff time in case of injury. The time to elicit a paw withdrawal or lick was recorded as the paw withdrawal latency. This was repeated 3 times in 5 min intervals. The average latency value was calculated and used for analysis.

2.6. Western blot

Immediately after the mechanical withdrawal threshold and thermal withdrawal latency were measured, the rats' L5/6 spinal lumbar enlargements were collected, homogenized with a RIPA lysis buffer, and centrifuged at 4 °C, 12000 rpm for 5 min. The RIPA lysis buffer contains protease inhibitor phenylmethylsulfonyl fluoride (Beyotime Biotechnology, China). Protein concentrations of lysates were measured with a standard bicinchoninic acid protein assay (Beyotime Biotechnology, China). Then, the proteins were separated by SDS-PAGE (12%) and transferred to a PVDF membrane for immunostaining. The membrane was blocked by 5% skim milk with 0.1% Tween 20 at 25 °C for 60 min. The membrane was incubated with primary antibodies of P-p38 (1:1000, rabbit polyclonal, Cell Signaling Technology, Boston USA) with GAPDH