Brain Research Bulletin 106 (2014) 47–55

Contents lists available at ScienceDirect

Brain Research Bulletin

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

Research report

Hydrogen-rich saline controls remifentanil-induced hypernociception and NMDA receptor NR1 subunit membrane trafficking through GSK-3β in the DRG in rats

Linlin Zhang[a,b], Ruichen Shu[a,b], Chunyan Wang[a,b], Haiyun Wang[a,b], Nan Li[a,b], Guolin Wang[a,b,*]

[a] Department of Anesthesiology, Tianjin Medical University General Hospital, Tianjin 300052, PR China
[b] Tianjin Research Institute of Anesthesiology, Tianjin 300052, PR China

ARTICLE INFO

Article history:
Received 14 April 2014
Received in revised form 20 May 2014
Accepted 21 May 2014
Available online 18 June 2014

Keywords:
Hydrogen-rich saline
Opioid-induced hyperalgesia
NR1
GSK-3β
DRG

ABSTRACT

Background: Although NMDAR trafficking mediated by GSK-3β involvement in transmission of nociceptive messages in the spinal cord has been confirmed by our previous studies, whether NMDAR trafficking is implicated in peripheral sensitization remains equivocal. It is demonstrated that inflammation is associated with spinal NMDAR-containing nociceptive neurons activation and the maintenance of opioid induced pain hypersensitivity. However, whether and how hydrogen-rich saline, as an effective anti-inflammatory drug, could prevent hyperalgesia through affecting peripheral sensitization caused by NMDAR activation remains to be explored.

Methods: To test these effects, hydrogen-rich saline (2.5, 5 or 10 ml/kg) was administrated intraperitoneally after remifentanil infusion, NMDAR antagonist MK-801 or GSK-3β inhibitor TDZD-8 was administrated intravenously before remifentanil infusion in rats. We examined time course of hydrogen concentration in blood after hydrogen-rich saline administration. Mechanical and thermal hyperalgesia were evaluated by measuring PWT and PWL for 48 post-infusion hours, respectively. Western blotting and real-time qPCR assay were applied to analyze the NR1 membrane trafficking, GSK-3β expression and activity in DRG. Inflammatory mediators (TNF-α, IL-1β, and IL-6) expressions in DRG were also analyzed.

Results: We found that NR1 membrane trafficking in DRG increased, possibly due to GSK-3β activation after remifentanil infusion. We also discovered that hydrogen-rich saline not 2.5 ml/kg but 5 and 10 ml/kg could dose-dependently attenuate mechanical and thermal hyperalgesia without affecting baseline nociceptive threshold, reduce expressions of inflammatory mediators (TNF-α, IL-1β, and IL-6) and decrease NR1 trafficking mediated by GSK-3β, and minimal effective concentration was observed to be higher than 10 μmol/L, namely peak concentration in arterial blood after administration of HRS 2.5 ml/kg without any influence on hyperalgesia.

Conclusion: Our results indicated that antihyperalgesic effect of hydrogen-rich saline might depend predominantly on its ability to reverse NR1 trafficking via inhibition of GSK-3β activity in DRG in a dose-dependent manner.

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; COX, cyclooxygenase; DRG, dorsal root ganglion; EGFR, epidermal growth factor receptor; GAPDH, glyceraldehydes 3-phosphate dehydrogenase; GSK-3β, glycogen synthase kinase-3β; HRS, hydrogen-rich saline; NMDA, N-methyl-D-aspartate; NS, normal saline; NSAIDs, non-steroidal anti-inflammatory drugs; OIH, opioid-induced hyperalgesia; PWL, paw withdrawal thermal latency; PWT, Paw withdrawal mechanical threshold; SaO_2, arterial oxygen saturation; TNF-α, tumor necrosis factor-alpha.

* Corresponding author at: Department of Anesthesiology, Tianjin Medical University General Hospital, Tianjin 300052, PR China. Tel.: +86 15822855556; fax: +86 15822855556.
E-mail addresses: 570502453@qq.com (L. Zhang), shurui.chen@163.com (R. Shu), 0208wcy@163.com (C. Wang), wanghy819@hotmail.com (H. Wang), linanredstone@126.com (N. Li), wang_guolin@hotmail.com, 970237902@qq.com (G. Wang).

http://dx.doi.org/10.1016/j.brainresbull.2014.05.005
0361-9230/

OPIOIDS remain first-line analgesics available for moderate-to-severe analgesia and anesthesia, however, accumulating experimental and clinical researches demonstrated that opioids medication might result in opioid-induced hyperalgesia (OIH) (Lee et al., 2011; Liang et al., 2011; Ohnesorge et al., 2013). Numerous articles have manifested that remifentanil, a short-acting selective MOR agonist, at clinically relevant dose as an intraoperative analgesic could cause pain hypersensitivity and enhance postoperative analgesics consumption (Bornemann-Cimenti et al., 2012; Ishida et al., 2012).

It is demonstrated that N-methyl-D-aspartate (NMDA) receptor activation induced central sensitization exerts an irreplaceable influence on the initiation and maintenance of OIH (Gu et al., 2009; Li et al., 2013; Zhao and Joo, 2008). The level of NMDA receptors, particularly NR1, in primary sensory neurons increases during inflammation, which contributes to peripheral inflammatory pain sensitization (Du et al., 2003; Tan et al., 2010). Glycogen synthase kinase-3β (GSK-3β) plays a key regulatory role in NR1 membrane trafficking in the spinal cord after remifentanil infusion (Chen et al., 2007; Yuan et al., 2013). However, whether and how peripheral sensitization caused by NMDAR activation is implicated in OIH has been not reported.

Proinflammatory cytokines are associated with spinal NMDAR-containing nociceptive neurons activation and the maintenance of inflammatory pain (Weyerbacher et al., 2010; Ian et al., 2004; Zhang et al., 2008). It is convinced that inhalation of H_2 efficiently suppresses proinflammatory cytokines release (Huang et al., 2011; Ji et al., 2010), however, in clinical application, inhalation of H_2 gas is not convenient and is dangerous because of its flammable and explosive nature even at a concentration of 4.7% in air (Ohsawa et al., 2007). In contrast to H_2 gas, hydrogen-rich saline (HRS) is easily administered and safe for application (Ji et al., 2011; Li et al., 2010). Simultaneously, inhibition of GSK-3β activity also attenuates the expression of proinflammatory cytokines to suppress inflammation (Chen et al., 2012). However, whether and how anti-inflammation of HRS prevents inflammatory hypernociception via affecting peripheral sensitization caused by NMDAR activation mediated by GSK-3β remains to be explored.

In the current study, we aimed to test the hypothesis that OIH involved NMDA receptor membrane trafficking through GSK-3β pathway and HRS might reverse hyperalgesia and decrease NMDA receptor trafficking through suppression of GSK-3β activity in dorsal root ganglion (DRG) in a rat model of remifentanil-induced hyperalgesia. To test these effects, HRS was administrated intraperitoneally after remifentanil infusion, NMDA antagonist MK-801 or GSK-3β inhibitor TDZD-8 was administrated intravenously before remifentanil infusion.

1. Materials and methods

1.1. Animals

Adult male Sprague-Dawley rats (250 g), purchased from the Laboratory Animal Center of the Military Medical Science Academy of the Chinese People's Liberation Army, were used throughout all experiments and housed under 12 h light/dark cycles with free access to water and food. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Tianjin Medical University and were in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and the number of animals used.

1.2. Preparation of hydrogen-rich saline

HRS was prepared as described by Li et al. (2010) Hydrogen was dissolved in normal saline (NS) for 6 h under high pressure (0.4 MPa) to a supersaturated level using a hydrogen-rich water producing apparatus (YUTAKA Engineering Co., Tokyo, Japan). The saturated hydrogen saline was stored under atmospheric pressure at 4 °C in an aluminum bag without dead volume, sterilized by gamma radiation and freshly prepared every week to ensure a constant concentration higher than 0.6 mmol/L.

1.3. Experimental protocols

Rats were anesthetized with sevoflurane (induction, 3.0%; surgery, 1.0%; batch number: 100628; Maruishi Pharmaceutical Co., Osaka, Japan) by a nose mask under sterile conditions. This study consisted of two experiments. Experiment 1 was conducted to elucidate whether HRS with different doses could attenuate remifentanil induced postinfusion hyperalgesia through decrease in NMDAR membrane trafficking in DRG. Experiment 2 was undertaken to determine whether suppression of NMDAR membrane trafficking in DRG after HRS administration was associated with GSK-3β.

1.3.1. Experiment 1

80 rats were randomly divided into ten groups ($n = 8$ each group): group C (NS 0.1 ml kg^{-1} min^{-1} for 60 min, iv); group C+ H1, C+ H2, C+ H3 (intraperitoneal injection of HRS 2.5 ml/kg, 5 ml/kg, 10 ml/kg at the end of NS infusion); group R (remifentanil infusion); group H1, H2, H3 (intraperitoneal injection of HRS 2.5 ml/kg, 5 ml/kg, 10 ml/kg at the end of remifentanil infusion); group M (MK801 0.1 mg/kg); group M + H1 (MK801 0.1 mg/kg, HRS 2.5 ml/kg). Remifentanil hydrochloride (batch number 090907; RenFu Co., Yichang, China) was dissolved in NS and infused via caudal vein at a rate of 1 μg kg^{-1} min^{-1} for 60 min. MK801 (a NMDAR antagonist, Sigma, Canada) was injected intravenously before remifentanil infusion. Paw withdrawal mechanical threshold (PWT) and paw withdrawal thermal latency (PWL) were measured at 24 h before and 2, 6, 24 and 48 h after remifentanil or NS administration. The L_4–L_6 segments of DRG were collected after finishing behavioral testings for determining inflammatory mediators (TNF-α, IL-1β, and IL-6) expression, NR1 membrane trafficking, the level of GSK-3β mRNA and protein, and phosphorylation (Ser9) of GSK-3β.

1.3.2. Experiment 2

48 rats were randomly divided into six groups ($n = 8$ each group): C group (NS, iv); R group (remifentanil infusion); T group (TDZD-8, 1 mg/kg); T1 group (TDZD-8, 0.5 mg/kg); T1+ H1 group (TDZD-8, 0.5 mg/kg; HRS, 2.5 ml/kg) and H1 group (HRS, 2.5 ml/kg). TDZD-8 (a GSK-3β inhibitor, Sigma, Canada) was administrated intravenously before remifentanil infusion. HRS was administrated intraperitoneally after remifentanil infusion. Behavioral tests, NR1 membrane trafficking, and GSK-3β activity were measured as experiment 1.

1.4. Measurement of hydrogen concentration in blood

Molecular hydrogen in artery and vein was measured using a needle-type Hydrogen Sensor (Unisense A/S, Aarhus, Denmark) following the method published (Hayashida et al., 2008). Arterial and venous blood were collected and taken at 5 min before and 5, 10, 15, 20, 30, 45 and 60 min after intraperitoneal administration of HRS.

1.5. Analysis of arterial blood gas

Blood samples were collected for gas analysis using a clinical blood-gas analysis device (GEM Premier3000; Instrumentation Laboratory, The Netherlands). PH, PaO_2, $PaCO_2$, and SaO_2 (arterial