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Research report

The $P2X_7$ receptor in dorsal root ganglia is involved in HIV gp120-associated neuropathic pain

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ABSTRACT

Human immunodeficiency virus (HIV)-associated neuropathic pain is common, and studies have shown that HIV envelope glycoprotein 120 (gp120) can directly stimulate primary sensory afferent neurons causing hyperalgesia. The $P2X_7$ receptor in the dorsal root ganglia (DRG) is involved in pain transmission and is closely related to the inflammatory and immune response. In this study, we aimed to explore the role of the $P2X_7$ receptor in gp120-induced neuropathic pain using a rat model specific for this type of pain. The results showed that mechanical hyperalgesia, thermal hyperalgesia and $P2X_7$ expression levels were increased in rats treated with gp120. The $P2X_7$ antagonist, brilliant blue G (BBG), decreased hyperalgesia and $P2X_7$ expression levels in rats treated with gp120. BBG also decreased IL-1β and TNF-α receptor expression and ERK1/2 phosphorylation levels and increased IL-10 expression in the gp120-treated rat DRG. In addition, $P2X_7$ agonist (BzATP)-activated currents in DRG neurons cultured with gp120 were larger than those in control neurons, and the inhibitory effect of BBG on BzATP-induced currents in gp120-treated DRG neurons was larger than that in control neurons. Therefore, inhibition of the $P2X_7$ receptor in rat DRG relieved gp120-induced mechanical hyperalgesia and thermal hyperalgesia.

1. Introduction

Chronic pain is a common symptom in people living with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), affecting > 60% of HIV-1-infected patients (Hao, 2013; Parker et al., 2014; Schutz and Robinson-Papp, 2013). Chronic pain dramatically reduces the quality of life of HIV-1/AIDS patients and is one of the primary reasons patients seek medical assistance (Kamerman et al., 2012; Keltner et al., 2014; Maratou et al., 2009; Verma et al., 2005). HIV-1 proteins have been shown to induce pain behaviors when introduced into animal models; glycoprotein 120 (gp120) is an HIV-1 protein that may cause HIV-associated neuropathic pain (Hao, 2013; Nasirinezhad et al., 2015; Yuan et al., 2014; Zheng et al., 2011). Distal symmetrical polyneuropathy (DSP) is one of the most common neurologic complications associated with HIV (Maratou et al., 2009; Schutz and Robinson-Papp, 2013). The dorsal root ganglia (DRG) transmit noxious stimuli from the periphery to the central nervous system and DRG afferent fibers are distributed to both central and peripheral terminals (Basbaum et al., 2009). Complications in HIV-associated neuropathic pain also include spontaneous pain and evoked pain (Freeman et al., 2014; Verma et al., 2005). Peripheral administration of gp120 enhances thermal hyperalgesia and mechanical allodynia in rats (Hao, 2013; Herzberg and Sagen, 2001; Kamerman et al., 2012; Maratou et al., 2009; Milligan et al., 2000; Oh et al., 2001; Wallace et al., 2007). HIV-1 gp120 interactions with the peripheral nerve may be involved in the generation of peripheral neuropathic pain in humans. The understanding of how HIV-1 gp120 leads to chronic pain is essential for the development of effective therapy.

Adenosine triphosphate (ATP) is thought to be a crucial molecule in acute pain signaling in the DRG, as well as in the development and maintenance of chronic pain (Burnstock, 2009, 2013, 2014; Chizh and Illes, 2001). Extracellular ATP activates the ionotropic P2X receptors (Burnstock, 2009, 2013, 2014; Chizh and Illes, 2001; Khakh and North, 2012), which are expressed by neurons of the DRG (Burnstock, 2009). The $P2X_7$ receptor has important functions in the immune system (Burnstock, 2013; Mehta et al., 2014; Skaper et al., 2010); studies show

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that the severity of the inflammatory response is decreased in $P2X_7$ receptor gene knockout phenotypes (Mehta et al., 2014; Skaper et al., 2010). The $P2X_7$ receptor is important for the induction and maintenance of neuropathic and inflammatory pain (Burnstock, 2013; Liu et al., 2015; Skaper et al., 2010; Sperlagh et al., 2006). It was reported that the interaction of the HIV surface protein gp120 with macrophages stimulates increased ATP release (Hazleton et al., 2012; Lee et al., 2012). P2X receptors are required for HIV entry into macrophages (Hazleton et al., 2012). ATP signaling via the $P2X_7$ receptor plays a key role in the regulation of inflammatory responses during acute viral infection (Lee et al., 2012). It is possible that the $P2X_7$ receptor is involved in HIV-associated neuropathic pain. However, no studies have been performed to assess the effects of the $P2X_7$ receptor on HIV gp120-associated pain behaviors and neuropathology in a rat model. In this study, we investigated the effects of the $P2X_7$ receptor and its antagonist on pain behaviors and gp120-induced neuropathology in rat primary afferent fibers.

2. Materials and methods

2.1. Animals and surgical methods

Male Sprague-Dawley (SD) rats weighing 200–250 g were used in all experiments. The rats were housed in separate solid-bottom cages with wood shavings for bedding. All rats had *ad libitum* access to drinking water and standard rat chow. The lighting was set on a 12 h light-dark cycle with lights from 7:00 and ambient temperature was set at 21 °C and a relative humidity range of 40–50%. The rats were habituated to the housing conditions as well as the experimental interventions (handling and weighing) for three days before starting the experimental protocol. The use of the animals was reviewed and approved by the Animal Care and Use Committee of the Medical College of Nanchang University. The experiments were performed in accordance with the Guidelines of the National Institutes of Health in the US regarding the care and use of animals for experimental procedures.

The rats were randomly divided into three groups (with 10 rats in each group): the HIV-gp120 group (gp120 group); HIV-gp120 rats treated with the $P2X_7$ receptor antagonist, brilliant blue G (BBG; Sigma) (gp120 + BBG group); and the sham operation group (sham group). A previously described technique (Wallace et al., 2007) was used for perineural HIV-gp120 administration. Briefly, under 10% chloral hydrate anesthesia (3 mL/kg, i.p., supplemented as necessary), the left sciatic nerve of the SD rat was exposed in the popliteal fossa without damaging the nerve construction. A 2 × 6 mm strip of oxidized regenerated cellulose was soaked in 250 μL of a 0.1% rat serum albumin (RSA) saline solution containing 200 ng of gp120 (Sigma) or 0.1% RSA in saline for the sham surgery. Gp120 Fragment 421–438 which was used was derived from the CD4 attachment region of HIV gp120. A 3–4 mm length of the sciatic nerve proximal to the trifurcation was wrapped loosely with the soaked cellulose, avoiding nerve constriction, and was left in situ. The incision was closed with 4/0 sutures. Beginning at 24 h after surgery, the rats in the gp120 + BBG group were intraperitoneally administered BBG (30 mg/kg; dissolved in saline at a final concentration of 15 mg/mL) daily for 14 days (Tu et al., 2013). Meanwhile, the rats in the sham group and gp120 group were given the same volume of normal saline.

2.2. Measurement of the mechanical withdrawal threshold

Determination of the mechanical withdrawal threshold (MWT) was performed at 8:00–12:00 using a BME-404 electronic mechanical stimulator (Institute of Biomedical Engineering, Chinese Academy of Medical Sciences, Tianjin, China). The main technical parameters of this equipment were as follows: end face diameter of test needle, 0.6 mm; pressure measurement range, 0.1–50 g; and a pressure measurement resolution, 0.05 g. An organic glass box (22 × 22 × 12 cm) was placed on the sieve of the metal frame. The rat was placed into the box for a 30 min adaptation. The left hind paws were touched with the test needle until escape behavior appeared. The pressure value was automatically recorded. The measurement was conducted five times for each rat (interval, ≥5 min), and the mean value was calculated as the MWT (Lin et al., 2010; Nasirinezhad et al., 2015; Zhang et al., 2013). The test was performed in blind with a separate experimenter. Baseline measurements were obtained for all rats on day 0, 1, 4, 7, 10, 12, 14 after surgical. The baseline was calculated from an average of 5 consecutive withdrawal responses of the left hindpaw. All thresholds were measured by a 'blinded' observer. The threshold value at each time point tested was calculated as the mean ± SEM.

2.3. Measurement of the thermal withdrawal latency

The Thermal Paw Stimulation System (BME-410C, Tianjin) was used to determine Thermal withdrawal latency (TWL) (Lin et al., 2010; Nasirinezhad et al., 2015; Zhang et al., 2013). The animals were placed in the same box above described on a glass plate. After 0.5 h for adaptation, the plantar surface of their paws was exposed to a beam of radiant heat, which would be switched off when withdrawal responses of left hind paws appeared. The time on the screen was designated as the paw withdrawal latency. The hind paws were tested alternately at 5 min intervals. The cutoff time for heat stimulation was 30 s.

2.4. Real-time PCR

The rats in three groups were anesthetized by 10% chloral hydrate (3 mL/kg, i.p.). The left side of L4–6 DRG was isolated immediately and flushed with ice-cold PBS. The total RNA samples were prepared from the L4–6 DRG of each group using the TRIzol Total RNA Reagent (Beijing Tiangen Biotech Co.). cDNA synthesis was performed with 2 μg total RNA using the RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas, Burlington, Ontario, Canada). The primers were designed with Primer Express 3.0 software (Applied Biosystems Inc., Foster City, CA), and the sequences were as follows: $P2X_7$, forward 5′-GAGTCCGAGGCAATCTAATG-3′; reverse 5′-CTGTGATCCCAACAAAGG TC-3′; β-actin, forward 5′-TAAAGACCTCTATGCCAACACAGT-3, reverse 5′-CACGATGGAGGGGCCGGACTCATC-3′. Quantitative PCR was performed using the SYBR® Green MasterMix in an ABI PRISM® 7500 Sequence Detection System (Applied Biosystems Inc.). The quantification of gene expression was performed using the ΔΔCT calculation with CT as the threshold cycle. The relative levels of target genes, normalized to the sample with the lowest CT, were given as $2^{-\Delta\Delta CT}$ (Tu et al., 2013).

2.5. Western blotting

The animals were anesthetized and tissue collection was performed as described above, except that the tissues were snap-frozen in tubes on dry ice during the collection (Lin et al., 2010). Briefly, on the 14th day after the operation, the animals were anesthetized with chloral hydrate and the left side of L4–6 DRG was dissected. The DRG were isolated immediately and rinsed in ice-cold phosphate-buffered saline (PBS). The ganglia were homogenized by mechanical disruption in lysis buffer containing the following: 50 mM Tris-Cl, pH 8.0, 150 mM NaCl, 0.1% sodium dodecyl sulfate (SDS), 1% Nonidet P-40, 0.02% sodium deoxycholate, 100 μg/mL phenylmethylsulfonyl fluoride, and 1 μg/mL Aprotinin. The cells were incubated on ice for 50 min. The homogenates were then centrifuged at 12,000 rpm for 10 min and the supernatants were collected. The quantity of total proteins in the supernatants was determined using the Lowry method. After dilution with loading buffer (250 mM Tris-Cl, 200 mM Dithiothreitol, 10% SDS, 0.5% Bromophenol Blue, and 50% Glycerol) and heating to 95 °C for 5 min, samples containing equal amounts of protein (20 μg) were separated by 10% SDS–polyacrylamide gel electrophoresis using a Bio-Rad system. The