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Full-length Article

The effects of dexmedetomidine pretreatment on the pro- and anti-inflammation systems after spinal cord injury in rats

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ABSTRACT

Excessive inflammatory responses play important roles in the aggravation of secondary damage to an injured spinal cord. Dexmedetomidine (DEX), a selective α2-adrenoceptor agonist, has recently been implied to be neuroprotective in clinical anesthesia, but the underlying mechanism is elusive. As signaling through Toll-like receptor 4 (TLR4) and nicotinic receptors (nAChRs, notably α7nAChR) play important roles in the pro- and anti-inflammation systems in the central nervous system, respectively, this study investigated whether and how they were modulated by DEX pretreatment in a rat model of spinal cord compression. The model was used to mimic perioperative compressive spinal cord injury (SCI) during spinal correction. DEX preconditioning improved locomotor scores after SCI, which was accompanied by increased α7nAChR and acetylcholine (Ach, an endogenous ligand of α7nAChR) expression as well as PI3K/Akt activation. However, there was a decrease in Ly6h (a negative regulator for α7nAChR trafficking), TLR4, PU.1 (a critical transcriptional regulator of TLR4), HMGB1 (an endogenous ligand of TLR4), and caspase 3-positive cells, which was prevented by intrathecal preconditioning with antagonists of either α2R, α7nAChR or PI3K/Akt. In addition, application of an α7nAChR agonist produced effects similar to those of DEX after SCI, while application of an α7nAChR antagonist reversed these effects. Furthermore, both α7nAChR and TLR4 were mainly co-expressed in NeuN-positive cells of the spinal ventral horn, but not in microglia or astrocytes after SCI. These findings imply that the α2R/PI3K/Akt/Ly6h and α7nAChR/PI3K/Akt/PU.1 cascades are required for upregulated α7nAChR and downregulated TLR4 expression by DEX pretreatment, respectively, which provided a unique insight into understanding DEX-mediated neuroprotection.

1. Introduction

Perioperative spinal cord injury (SCI) remains a devastating complication, leading to long lasting paraplegia, for which there is no effective prophylactic agent. Clinically, SCI commonly occurs during surgical correction of scoliosis (Auerbach et al., 2016) and thoracoabdominal aortic interventions (Bell et al., 2012), despite the standardized application of intraoperative neuromonitoring. In addition to ischemic injury (38%), cord compression (15%) is the most commonly cited source of injury during the intraoperative period (Auerbach et al., 2016). To mimic cord compression, we used a rat model of spinal cord clip-compression injury in the present study (Forgione et al., 2014).

SCI is characterized by initial and delayed injuries to the spinal cord, of which a delayed inflammatory response plays an important role in contributing to the severity of SCI (Bao et al., 2009; Fleming et al., 2006; Su et al., 2010; Valenzuela et al., 2012; Weishaupt et al., 2010). Toll-like receptors (TLRs) are well known for their important role in mediating the pro-inflammatory reaction associated with tissue insult, including the injured spinal cord (Heiman et al., 2014). Among their subtypes, TLR4 has been reported to be involved in facilitating neuronal death and demyelination (Alfonso-Loeches et al., 2012; Rosenberger et al., 2015; Schonberg et al., 2007) in various neuropathological disorders, as well as in increased damage to an injured spinal cord (Austin et al., 2012; Bell et al., 2014; Freeman et al., 2015), upon recogni-

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tion of endogenous ligands, such as high mobility group box-1 (HMGB1) (Lotze and Tracey, 2005; Yu et al., 2006).

Nicotinic acetylcholine receptors (nAChRs) are known for their important roles in modulating the cholinergic anti-inflammatory system (Damaj et al., 2014; Marrero and Bencherif, 2009; Wang et al., 2004). α7nAChR is one of the most investigated subtypes (D'Incamps and Ascher, 2014) and can interact with acetylcholine (Ach) released by the cholinergic pathway as well as reduce inflammation (Waldburger et al., 2008). Recently, the beneficial effects of α7nAChR have also been examined in the attenuation of hyperalgesia (Munro et al., 2012; Zhang et al., 2015), protection of ischemic brain and cultured neurons (Bertoni et al., 2014; Wang et al., 2012). The roles played by α7nAChR-mediated signaling in the protection of an injured spinal cord is largely unclear; however, this signaling pathway has shown positive effects on the downregulation of TLR4 expression in a sepsis model (Kim et al., 2013).

The anti-inflammatory effects of dexmedetomidine (DEX), a potent and selective α2-adrenoceptor (α2R) agonist, have been outlined in various pathological settings (Chi et al., 2015; Gu et al., 2011; Wu et al., 2013; Yao et al., 2015). Despite the detection of TLR4 downregulation mediated by DEX and α7nAChR intensification of the anti-inflammatory effects of DEX, the underlying mechanisms governing these effects in the injured spinal cord remain unclear.

Therefore, we investigated whether and how TLR4 and α7nAChR expression were modulated by DEX pretreatment in SCI rats to elucidate any contribution that they might have to the protective effects of DEX. In addition, the interaction between α7nAChR and TLR4 signaling was also determined in this regard. Because the PI3K/Akt pathway is a common downstream effector of α7nAChR and α2R signaling (Kim et al., 2013; Zhu et al., 2013) as well as an upstream regulator of the PU.1 (Kim et al., 2013) transcription factor, which belongs to the Ets family of transcription factors and is important for the transcriptional regulation of TLR4 (Park et al., 2013), and because Ly6h negatively regulates the trafficking of α7nAChR (Puddifoot et al., 2015), their possible roles were also examined.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (200–220 g) were supplied by the Laboratory Animal Center of the Drum Tower Hospital. Rats were allowed access to food and water ad libitum. All animal experiments were approved by the Nanjing University Animal Care Institute and in accordance with the guidelines of the National Institutes of Health.

2.2. SCI model

Moderate spinal cord injury was induced by conducting clip-compression injury on the spinal cord with an aneurysm clip as previously described (Forgione et al., 2014). Briefly, rats were anesthetized with pentobarbital sodium (60 mg/kg, i.p.). A laminectomy was performed around the T10 level. After the spinal cord was completely exposed, the aneurysm clip (Aesculap Yasargil, FT260T, Germany) was vertically employed to induce cord compression that lasted for 1 min at the T10 level. Sham-operated rats were subjected to laminectomy only.

After compression, the incision was closed, rats immediately received ceftazidime saline (6 mg/kg, s.c.) and were repeatedly injected for three days post-injury. Rats were treated with warming measures before recovery from anesthesia and were then caged individually with free access to food and water.

2.3. Drugs

Rats received intrathecal DEX (1, 2, and 4 μg/kg) or vehicle (saline) 30 min before SCI. A selective α7nAChR agonist, PHA-543613 (10 μg/20 μl, i.t.; Sigma); a selective α7nAChR antagonist, methyllycaconitine (MLA, 10 μg/20 μl, i.t.; Sigma) (Zhang et al., 2015); or a specific antagonist of α2R, atipamezole (20 μg/20 μl, i.t.; Sigma) (Xu et al., 1992), was dissolved in saline or 5% dimethyl sulfoxide (DMSO) and administered 5 min before (Chilton et al., 2004) DEX or saline treatment. A selective PI3K antagonist, LY294002 (10 μg/20 μl; Sigma), was dissolved in 5% DMSO and administered 5 min prior to DEX treatment. The dose of DEX and LY294002 treatment were used based on our preliminary studies. Administration of the drugs was performed in rats at the level of L5-L6, which is far from the injury site (T10), using direct transcutaneous intrathecal injection with no impairment on the rat's normal motor function (Mestre et al., 1994). Thus, the possibility of puncture injury to the spinal cord could be excluded.

2.4. Basso, Beattie, Bresnahan rating scale

The dynamic locomotor recovery after SCI was assessed using the Basso, Beattie, Bresnahan (BBB) scoring system (Basso et al., 1995), with ratings from 0 to 21 at the indicated time points before sacrifice.

2.5. Quantitative polymerase chain reaction

Total RNA was extracted from spinal cord tissue around the injury site (total 2 cm) using Trizol reagent (Invitrogen Life Technologies, 15596-026). A Revert Aid First Strand cDNA Synthesis Kit (Thermo, #K1622) was used to reverse transcribe the isolated RNA into cDNA. Quantitative polymerase chain reaction was performed using the Fast Start Universal SYBR Green Master (Rox) qPCR Mastermix (Roche, 04913914001). Samples were incubated at 55 °C for 5 min and 95 °C for 10 min, followed by 40 cycles of amplification reactions (95 °C for 15 s and 60 °C for 60 s). The rat primers for β-actin and TLR4 were purchased from Invitrogen Biotechnology Co., Ltd. (NM_031144; NM_019178, respectively) with the following sequences: rat β-actin, TGCTATGTTGCCCTAGACTTCG (sense; accession number: NM_031144.3), GTTGGCATAGAGGTCTTTACGG (anti-sense); TLR4, TGGCATCATCTTCATTGTCC (sense; accession number: NM_019178.1), CAGAGCATTGTCCTCCC (anti-sense). All final outcomes are presented as relative mRNA levels.

2.6. Western blot

Rats were sacrificed 3 d after the operation with deep pentobarbital (100 mg/kg; i.p.) anesthesia. Then, the spinal cord tissue around the penumbra (total 2 cm) was removed and rapidly stored at −80 °C. Proteins were extracted from tissue homogenates in lysis buffer (Sigma), followed by centrifugation at 13,000 rpm for 10 min at 4 °C. The supernatant was collected for Western blotting. We determined the protein concentration using a BCA Protein Assay Kit. Samples containing 70 μg of protein were fractionated on SDS-PAGE (6%) and then electro-transferred onto a nitrocellulose membrane (Sigma). Blots were blocked for 1 h, incubated in the appropriate primary antibodies overnight at 4 °C, and finally probed using horseradish peroxidase-conjugated secondary antibodies (Sigma). The intensity of the immunoreactive bands was visualized using enhanced chemiluminescence (Cell Signaling). Primary antibodies against α7nAChR (Rat monoclonal antibody; 1:500, Abcam, ab24644), TLR4 (Mouse monoclonal antibody; 1:500, Abcam, ab22048), PU.1 (Rabbit polyclonal antibody; 1:1000, Abcam, ab83399), cleaved caspase-3 (Rabbit monoclonal