BRAIN RESEARCH 1573 (2014) 27–36

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Brain Research

Research Report

Selective activation of α7 nicotinic acetylcholine receptor (nAChRα7) inhibits muscular degeneration in mdx dystrophic mice

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ARTICLE INFO

Article history:
Accepted 3 May 2014
Available online 14 May 2014

Keywords:
Skeletal muscle
Mdx mice
Muscular dystrophy
Inflammation
Acetylcholine receptor
Cholinergic anti-inflammatory pathway

ABSTRACT

Amount evidence indicates that α7 nicotinic acetylcholine receptor (nAChRα7) activation reduces production of inflammatory mediators. This work aimed to verify the influence of endogenous nAChRα7 activation on the regulation of full-blown muscular inflammation in mdx mouse with Duchenne muscular dystrophy. We used mdx mice with 3 weeks-old at the height myonecrosis, and C57 nAChRα7$^{+/+}$ wild-type and nAChRα7$^{-/-}$ knockout mice with muscular injury induced with 60 μL 0.5% bupivacaine (bp) in the gastrocnemius muscle. Pharmacological treatment included selective nAChRα7 agonist PNU282987 (0.3 mg/kg and 1.0 mg/kg) and the antagonist methyllycaconitine (MLA at 1.0 mg/kg) injected intraperitoneally for 7 days. Selective nAChRα7 activation of mdx mice with PNU282987 reduced circulating levels of lactate dehydrogenase (LDH, a marker of cell death by necrosis) and the area of perivascular inflammatory infiltrate, and production of inflammatory mediators TNFα and metalloprotease MMP-9 activity. Conversely, PNU282987 treatment increased MMP-2 activity, an indication of muscular tissue remodeling associated with regeneration, in both mdx mice and WTα7 mice with bp-induced muscular lesion. Treatment with PNU282987 had no effect on α7KO, and MLA abolished the nAChRα7 agonist-induced anti-inflammatory effect in both mdx and WT. In conclusion, nAChRα7 activation inhibits muscular inflammation and activates tissue remodeling by

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http://dx.doi.org/10.1016/j.brainres.2014.05.004
0006-8993/

increasing muscular regeneration. These effects were not accompanied with fibrosis and/or deposition of non-functional collagen. The nAChRα7 activation may be considered as a potential target for pharmacological strategies to reduce inflammation and activate mechanisms of muscular regeneration.

1. Introduction

The nervous and immune systems are not fully independent. In order to maintain homeostasis, both produce cytokines, neurotransmitters and/or express receptors that act upon important physiological functions (Gallowitsch-Puerta and Pavlov, 2007; Junger, 2011; Pena et al., 2011; Rosas-Ballina et al., 2011; Tracey, 2002; Ulloa, 2013; Wang et al., 2004). The vagus nerve system (VNS) exerts an essential role in the regulation of inflammation via releasing endogenous acetylcholine (ACh) in the parenchyma of innervated organs. Although predominantly expressed in neuronal tissues, several types of immune cells, including macrophages, also express nAChRα7 mRNA (AlSharari et al., 2013), and reduction of inflammatory cytokine production often occurs following nAChRα7 macrophage signaling (Bernik et al., 2002). Moreover, in vitro treatment of lipopolysaccharide-stimulated human macrophages with ACh reduces production of pro-inflammatory cytokines (TNFα, IL-1β, IL-6, IL-18) but not of the anti-inflammatory cytokine IL-10, and direct in vivo VNS stimulation also prevented development of shock (Borovikova et al., 2000). Likewise, ACh released from activate spleen T lymphocytes interact with nAChRα7 on macrophages present in the red pulp and marginal zone inhibiting TNFα production (Andersson and Tracey, 2012; Rosas-Ballina et al., 2011) and B cell antibody secretion (Andersson and Tracey, 2012), but activating the release of the anti-inflammatory IL-10 cytokine from Th2 cells (Trakhtenberg and Goldberg, 2011).

The potential role of the cholinergic anti-inflammatory pathway via VNS and nAChRs activation has been demonstrated in various experimental models of septic peritonitis (Borovikova et al., 2000); ultraviolet experimentally injured skin (Osborne-Hereford et al., 2008); collagen-induced rheumatoid arthritis (van Maanen et al., 2009), and more recently it was demonstrated that nAChRα7-dependent mechanisms and signaling are involved in the modulation of chronic inflammatory neuropathic pain (AlSharari et al., 2013), and also neuroprotection under ischemic conditions by regulation of neuroinflammation and oxidative stress (Parada et al., 2013).

Duchenne muscular dystrophy (DMD) is an X-linked progressive fatal myopathy caused by mutations in the gene encoding for the cytoskeletal protein dystrophin, which is important for organizing the membrane cytoskeleton, and aggregating ion channels and neurotransmitter receptors (Carlson, 1998; Hoffman et al., 1987). Lack of dystrophin compromises the structural integrity of the cell membrane leading to aberrant intracellular signaling cascades that regulate both inflammatory and immune activities and contribute substantially to the physiopathology of muscular lesion (Evans et al., 2009; Lagrota-Candido et al., 2002). Mdx mouse, the animal model of human DMD, develops a benign phenotype with a multi-staged disorder characterized by intense myonecrosis with scattered inflammatory infiltrate at 4 weeks (4 wks) of age followed by muscular regeneration (12 wks) and later persistent fibrosis (24 wks) (Collins and Morgan, 2003; Evans et al., 2009; Lagrota-Candido et al., 2002). Our group observed a modulation of nAChRα7 expression at different stages of the mdx muscular dystrophy, and that nicotine treatment attenuated muscular inflammation and increased muscle regeneration (Leite et al., 2010). This work aimed to provide additional insight on selective activation of nAChRα7 in the mdx muscular inflammation. For such purpose, we used the α7 KO mice and pharmacological manipulation (Parada et al., 2013) with PNU282987 and the nAChRα7 antagonist methyllycaconitine (MLA). This approach allowed us to determine a putative role of α7-mediated stimulation or blockade of endogenous cholinergic activation in the mdx muscular pathology.

2. Results

2.1. *Selective nAChRα7 activation reduces serum and muscular inflammation in mdx mice*

TNFα circulating levels were determined to verify a putative effect of nAChRα7 activation in mdx mice inflammation. For such purpose it was chosen a 7-daily treatment with PNU282987, a selective nAChRα7 agonist used at two different doses (0.3 and 1.0 mg/kg); the MLA antagonist (1.0 mg/kg), or both agonist and MLA at 1.0 mg/kg. Intraperitoneal treatment of mdx mice with low dose (0.3 mg/kg) PNU282987 reduced TNFα circulating levels (Fig. 1A), and such effect was even more evident (21±3.3%; $p<0.005$) following treatment with 1.0 mg/kg dose. Conversely, treatment of mdx mice with the competitive antagonist MLA at 1 mg/kg abolished the effect, being observed a slight increase on TNFα circulating levels comparing with vehicle-treated mouse. Furthermore, in combination with the agonist (1 mg/kg PNU282987 plus 1 mg/kg MLA) completely abolished the agonist effect, thus indicating a role for nAChRα7 in reducing the production of TNFα pro-inflammatory cytokine.

Next, we verified whether nAChRα7 activation could influence in the production and activity of MMPs, a family of endopeptidases which are important in normal physiological processes and skeletal tissue remodeling (Bani et al., 2008). Increased MMP-9 activity in the serum reflects a process of active ongoing inflammation in several models (Khandoga et al., 2006; Maddahi et al., 2012; Schiøtz Thorud et al., 2005). Treatment with 1 mg/kg PNU282987 caused a marked reduction (91±16%; $p<0.005$) of MMP-9 activity in the sera and also a significant reduction (54±18%, $p<0.05$) in the gastrocnemius