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Advanced age enhances the sepsis-induced up-regulation of the γ- and α7-nicotinic acetylcholine receptors in different parts of the skeletal muscles

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ABSTRACT

Background: The muscle mass decreases with age, leading to frailty and weakness; however, the role of acetylcholine receptors in this process has not been properly studied. In this article, we hypothesize that diaphragmatic as well as peripheral muscle weakness may be caused by the up-regulation of gamma and alpha 7 nicotinic acetylcholine receptors (nAChRs) on muscle cell membranes.
Method: Adult male rats were randomly divided into sham and sepsis groups. Sepsis was induced by cecal ligation and puncture (CLP). Blood specimens and biopsies of tibialis anterior muscle and diaphragm were obtained at 24 h post CLP.
Results: Up-regulation of gamma and alpha 7 nAChRs was detected in both sham and septic groups; however, this response was more robust in septic animals. Compared to tibialis anterior muscle, the diaphragm expressed a higher number of both receptor types.
Conclusions: Muscle weakness in old age and sepsis may have common molecular underpinnings. Loss of diaphragmatic strength may explain hypoxia and respiratory failure often encountered in frail elderly.

1. Introduction

Sepsis is a clinical syndrome caused by inflammation (Levy et al., 2003), in which patients frequently develop skeletal muscle dysfunction that is manifested as acquired muscle weakness, a reduced capacity to continue muscle contractions, and other neuropathies (Aare et al., 2012; Fredriksson et al., 2006). Similar phenomena are observed in many elderly patients who do not develop sepsis (Cacciani, Ogilvie, & Larsson, 2014), which emphasizes the fact that the underlying mechanisms of this disease are unclear.

Sepsis not only produces dysfunction in the limb muscles but also causes phrenic nerve neuropathy in elderly patients (Fredriksson et al., 2006), which suggests that there could be a different mechanisms of dysfunction in these different muscles. The phenotypes of this disease include low levels of plasma binding proteins and dysfunction of the microcirculation and vital organs (Nakayama et al., 2000; Narimatsu, Niiya, Kawamata, & Namiki, 2005; Nayci et al., 2005; Roberts, Pea, & Lipman, 2013).

Historically, few studies have focused on the role of nicotinic acetylcholine receptors (nAChR) in sepsis. Three variants of nAChRs have been identified in the post-junctional synapse: adult (ε-nAChR), fetal (γ-nAChR), and the neuronal α7 type (α7-nAChR). Normally, ε-nAChR is confined to the neuromuscular junctions (NMJ) in healthy adult patients (Issa et al., 2005). Denervation leads to the re-expression of γ- and α7-nAChR (Cope &Hunter, 2003; Fischer, Reinhardt, Albuquerque, & Maelicke, 1999); various pathological states (e.g., burns, denervation, and sepsis) can also induce γ- and α7-nAChR expression (Apel et al., 2009). This re-expression under non-denervation conditions is called "denervation-like" affection (Carlson et al., 2002). Our previous study showed that the pharmacodynamic changes in NDMRs that occur in the septic state are associated with the up-regulation of γ- and α7-nAChR (Liu et al., 2014); however, it remains unknown whether the skeletal muscle dysfunction is associated with the up-regulation of the nAChRs in elderly persons with sepsis.

Based on the above phenomena and conclusions, in this study, we aimed to test the hypothesis that advanced age could decrease the function of the skeletal muscles by up-regulating γ- and α7-nAChR expression. We used a typical rat model of sepsis that was induced by cecal ligation and puncture (CLP) to examine the effect of advanced age on the muscular function and the changes in the composition of the nAChRs in the septic state. We compared

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the severity of the sepsis and general physiology at 24 h after the CLP and sham surgery. Then, we assessed the muscular function and the degree of γ- and α7-nAChR expression in the tibialis anterior muscle and the diaphragm muscle in the sham and septic rats. Finally, we assessed the cross-correlations between age and treatment (sham or CLP) regarding the expression of γ- and α7-nAChR in the skeletal muscles.

2. Materials and methods

2.1. Animals

Twenty male adult Sprague-Dawley (SD) rats (age: 5 months, weight range: 220–250 g) and 20 male aged SD rats (age: 20 months, weight range: 475–500 g) were acquired from the Experimental Animal Center of Chongqing Medical University (Chongqing, China). All rats received humane care according to the Animal Ethics and Use Committee of Chongqing Medical University. The rats were housed in a specific pathogen-free room under standardized conditions (25 ± 2 °C, 60% humidity and 12 h light/dark cycle) for one week before the experiment. The rats had free access to food and water. All animal manipulations were performed in accordance with the standards for the use of laboratory animals issued by the National Institute of Health and had been approved by the Animal Ethics and Use Committee of Chongqing Medical University.

2.2. Anesthesia and vital parameters

On the day of the experiment, we anesthetized the rats with an i.p. injection of 0.5% sodium pentobarbital (65 mg kg^{-1}) to prepare them for surgery. The animals were tracheotomized, and ventilated with a small animal ventilator (TKR-200C, Jiangxi Teli Anaesthesia & Respiratory Equipment Co., Ltd., Jiangxi Province, China). The mean arterial pressure, heart rate, and body temperature (°C) were continuously monitored (RM6240 Systems, Inc., Chengdu, China). The levels of PaO_2, $PaCO_2$ and the acid–base status were adjusted to ensure stable hemodynamic conditions. The rats were excluded from the experiment if they were hemodynamically unstable (mean arterial pressure < 10.7 kPa) or if their blood gas levels were not within the normal range [PaO_2 > 13.6 kPa; pH 7.35–7.45; $PaCO_2$ 4.67–6.00 kPa; base excess of −2 (2) mEq] (Liu et al., 2014).

2.3. Group assignments and animal models

The twenty male adult SD rats were randomly divided into 2 subgroups of 10 rats each: (i) the sepsis group underwent cecal ligation and puncture (CLP) (CLP-Adult, $n = 10$); and (ii) the sham group underwent a sham operation (Sham-Adult, $n = 10$). Likewise, the twenty male aged SD rats were randomly divided into the CLP-Aged subgroup and the Sham-Aged subgroup (10 rats in each subgroup).

We established a model of sepsis, as previously described (Rosenheimer, 1990). In this model, the rats were considered to have acute sepsis after 24 h upon completion of the CLP (Xiao, Siddiqui, & Remick, 2006; Zanotti-Cavazzoni & Goldfarb, 2009). We anesthetized the rats with an i.p. injection of 0.5% sodium pentobarbital (65 mg kg^{-1}). We used tail clamping to assess consciousness and determine a sufficient depth of anesthesia; we then weighed the rats and placed them on a heating pad in the supine position. After sufficient anesthesia was established, the abdomens of the rats were shaved and cleaned before performing a 2 cm long incision through the skin and rectus abdominis on the left side of the midline to expose the cecum. We located and carefully exteriorized each rat's cecum, and then ligated it with a 3–0 silk suture halfway from the base of the ileocecal valves. Subsequently, the ligated cecum was punctured with a 24-gauge needle twice to make sure the wounds were infected with a small amount of stool. Finally, the cecum was carefully returned to the abdomen. The abdomen was closed with a 3–0 silk suture. In the sham group, the cecum was returned to the abdomen after gentle manipulation. In all groups, 10 mL kg^{-1} of 0.9% normal saline was subcutaneously injected after the abdomen was closed to restore the animals' fluid levels.

2.4. Serum cytokine measurements

The serum cytokine levels were used to measure the severity of the sepsis in the surviving rats. Twenty-four hours after the CLP operation, the rats were deeply anesthetized with sodium pentobarbital (0.5%, 65 mg kg^{-1}, ip). We collected the blood samples immediately after decapitation and stored them on ice until the serum was prepared. The samples were centrifuged at 2000 × g for 15 min to separate the serum, then stored at −80 °C before measuring the interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) levels. The cytokine concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits (both kits were from CUSABIO BIOTECH Co., Ltd., China) according to the manufacturer's instructions.

2.5. Evaluation of muscular functions

All of the surviving rats were deeply anaesthetized with 0.5% sodium pentobarbital (65 mg kg^{-1}, i.p.) 24 h after the CLP and sham surgeries. The fresh diaphragm (breadth less than 5 mm) and tibialis anterior muscle (length less than 2 cm) samples were carefully harvested 24 h after surgery. All the results were recorded by researchers who were blinded to the experimental group. The samples were first balanced in Krebs' solution for 10 min and were then separately stimulated (intensity 3 V, duration 0.2 ms and frequency 1 Hz) by an electrode (RM6240 Systems, Inc., Chengdu, China) to define the optimum contractile length (L_0). The sampled muscles were first stimulated with different frequencies (10, 20, 40, 60, 80, 100 and 120 Hz) of interval twitches (intensity 3 V, duration 0.2 ms), with the time interval set at 2 min. The peak twitch tension was recorded to construct a frequency–tension curve. Then, the sampled muscles were stimulated with a sustained stimulation (intensity 3 V, frequency 40 Hz) for 330 ms, paused for 670 ms, and cycled every 5 min; a tetanizing stimulation (for 1, 2, 3, 4, 5 and 6 min) was used to construct a fatigue–tension curve. All muscle samples were weighed after the experiments. The results were standardized using the following formula for calculating the divided cross sectional area (CSA) (Zhang, Yang, & Li, 2014):

$$\mathrm{CSA} = \frac{\mathrm{weight}}{L_0} \times 1.056$$

2.6. Tissue preparation

The tibialis anterior muscle and diaphragm muscle specimens were collected in ice-cold phosphate-buffered saline ([PBS], pH 7.4) and transferred to 4% paraformaldehyde after the muscular function evaluations were complete. After a 2 day fixation, the muscle specimens were then embedded in paraffin. The specimens were cut into 4 μm thick sections on a rotary microtome (RM2135, Leica Instruments, Germany) and placed onto glass slides. The sections were deparaffinized in dimethylbenzene, rehydrated successively in a gradient of ethanol and washed with distilled