Kaohsiung Journal of Medical Sciences (2018) 34, 301–306

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.kjms-online.com

Short Communication

Increased frequency of AMP-activated protein kinase-positive spinal motor neurons after sciatic nerve injury in a mouse model

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Received 15 September 2017; accepted 11 December 2017
Available online 12 January 2018

KEYWORDS
Sciatic nerve injury;
Motor neuron;
AMP-Activated protein kinase

Abstract The role of AMP-activated protein kinase (AMPK) in the regulation of energy metabolism and the control of skeletal muscle regeneration post injury has been described previously. It remains unknown whether this metabolic sensor plays a role in the mechanism of axonal regeneration post injury. In this study, we used a sciatic nerve crushed mouse model to detect the expression of AMPK in sciatic nerve and spinal motor neurons at 1 week, 2 weeks and 3 weeks after injury by immunofluorescence staining. Electrophysiological and histopathological studies were used to confirm the nerve injury and regeneration. Our results showed that frequency of AMPK-positive spinal motor neurons was significantly higher on day 7 after sciatic nerve crush (SNC) and peaked on day 14. No expression of AMPK was detected in axons of the sciatic nerve before and after the injury. Taken together, our study suggested a possible role of AMPK in the mechanism of motor nerve regeneration after injury.

Conflict of Interest: All authors declare no conflicts of interests.

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https://doi.org/10.1016/j.kjms.2017.12.007

Introduction

Traumatic peripheral nerve injury is common and may result in limb weakness and loss of sensory function. Investigating the mechanisms involved in nerve repair and regeneration post injury is necessary to improve nerve regeneration and therefore function. Generally, after peripheral nerve injury, Wallerian degeneration firstly occurs, followed by migration of activated macrophages to clear the myelin and axon debris and axonal sprouting from spinal neurons [1,2].

AMPK is a heterotrimeric serine/threonine kinase that exists in all eukaryotic cells and consists of a catalytic α subunit and two regulatory β and γ subunits [3,4]. Accumulating evidence indicates that AMPK plays an essential role in a series of neurophysiological and pathological processes [5,6]. Price et al. reported that AMPK activation could induce neuronal plasticity via mammalian target of rapamycin (mTOR) complex 1 [6]. AMPK signaling can inhibit tau phosphorylation and amyloid-beta production thereby delaying the progress of Alzheimer's disease in transgenic mice [7]. Furthermore, Gong et al. have demonstrated that some microRNA's function as a protector of dopaminergic neurons during Parkinson's disease by regulating the AMPK pathway [8]. However, it remains unknown whether AMPK participates in the mechanism of post injury nerve regeneration. In this study, using a sciatic nerve crushed (SNC) mouse model, we explored the dynamic expression of AMPK in spinal motor neurons during the nerve regeneration process.

Materials and methods

Animals

Male C57BL/6 mice (8—10 weeks) were purchased from Pengyue Laboratory Animal Breeding Limited Company (Jinan, China) and housed in a temperature-controlled facility with 12 h light/dark cycles and free access to food and water. The experiment was approved by the Institutional Animal Care and Use Committee of the Affiliated Hospital of Jining Medical University and performed in accordance with the Principles of Laboratory Animal Care.

Reagents

Phosphate-buffered saline (PBS, 0.1 M, PH 7.4) and Tween-20 were purchased from Boster (Wuhan, China). TritonX-100 was purchased from Genview (TX, USA). Goat serum was purchased from Zhongshan Golden Bridge Company (Beijing, China). Anti-neurofilament H nonphosphorylated antibody (SMI32) was purchased from Biolegend (CA, USA). Anti-AMPK alpha 1 + AMPK alpha 2 antibody, Alexa-568-conjugated goat anti-mouse IgG, Alexa-488-conjugated donkey anti-rabbit IgG and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Abcam (MA, USA).

Sciatic nerve crush

Twelve mice were randomly assigned to four groups (n = 3 per group): (1) normal control group, (2) SNC 7d group, (3) SNC 14d group and (4) SNC 21d group. The SNC group mice were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.05 ml/100 g). The left hindquarter was then carefully shaved prior to skin cleansing with Betadine swabs. The skin was incised 1 mm posterior and parallel to the femur, and the biceps femoris was bluntly split to expose the sciatic nerve. The sciatic nerve was then crushed 5 mm proximal to its trifurcation with No. 5 Jeweler's forceps for 30 s as previously described [9]. The nerve crush was confirmed by the presence of a translucent band across the nerve and electrophysiological test. The incision was then closed in layers (muscle and skin) with absorbable sutures to allow recovery. The contralateral sciatic nerve was left intact as a surgical control.

Electrophysiology study

The electrophysiological study was performed to confirm the crush model and monitor the process of nerve repair. An electrophysiological surveillance system was setup and nerve function was recorded prior to the operation and at 1 week, 2 weeks and 3 weeks postoperatively (before the tissue processing). The left sciatic nerve in each mouse was exposed at the hip (proximal), and then one pair of needle electrodes was inserted into the sciatic notch (hip/proximal). The nerve stimulation parameters used to elicit compound muscle action potential (CMAP) were: 1 Hz pulses, with each pulse being 5 mA in amplitude and 0.3 ms in duration. The recording electrodes were positioned in the "belly" part of the gastrocnemius muscle to record evoked potentials from stimulating the sciatic nerve. The distance between stimulating electrodes and recording electrodes was 1.5 cm. The nerve conduction velocity of the surviving axons was determined by dividing the distance between the stimulation and recording points and the latency between stimulation and the appearance of the action potentials (V = m/s). A heating pad was used to maintain the body temperature of the mouse at 37 °C.

Tissue processing

Mice at different time points were deeply anesthetized using 10% chloral hydrate (0.05 ml/100 g) and were perfused through the left heart ventricle with 40 ml of ice-cold PBS, followed by 40 ml of 4% paraformaldehyde in PBS. Then, sciatic nerve-originated L3 L4 spinal cords were identified as previously described [10] and the left sciatic nerves were removed carefully and post-fixed in 2% paraformaldehyde overnight at 4 °C. Half of the sciatic nerve from each mouse was processed for the electron microscopy analysis. Tissues were dehydrated with 30% sucrose solution in PBS. Cryostat sections of 10 μm thick were cut transversely for the immunofluorescence staining.

Electron microscopy

Sciatic nerves from different time points were fixed in a mixture of 2% paraformaldehyde and 3% glutaraldehyde in PBS overnight at 4 °C and the tissues were post-fixed using 1% osmium tetroxide in PBS for 1 h, dehydrated in a gradient alcohol series and immersed in propylene oxide