Neuroscience Letters 643 (2017) 103–110

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Neurons of the rat cervical spinal cord express vimentin and neurofilament after intraparenchymal injection of kainic acid

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HIGHLIGHTS

- Some spinal cord perikarya express VIM and NF after experimental injury.
- Expression of VIM and NF last for several days after injury.
- VIM and NF colocalize in the same perikarya after injury.
- VIM and NF expression may be a necessary change to promote recovery of the damaged tissue.

ARTICLE INFO

Article history:
Received 6 September 2016
Received in revised form 23 January 2017
Accepted 9 February 2017
Available online 14 February 2017

Keywords:
Intermediate filaments
Vimentin
Neurofilament
Kainic acid
Colocalization
Spinal cord

ABSTRACT

Intermediate filaments (IF) can be altered under disorders such as neurodegenerative diseases. Kainic acid (KA) induce behavioral changes and histopathological alterations of the spinal cord of injected rats. Our goal was to evaluate the IF expression in neurons during this injury model. Animals were injected with KA at the C5 segment of the cervical spinal cord and euthanized at 1, 3 and 7 post injection (pi) days. Neuronal cell counting showed a significant loss of neurons at the injection site when compared with those of sham and non-operated animals. Immunohistochemistry for vimentin and neurofilament showed positive labeling of perikarya in sham and KA-injected animals since day 1 pi that lasted for the remaining experimental days. Colocalization analysis between enolase and vimentin or neurofilament confirmed a high index of colocalization in both experimental groups at day 1 pi. This index decreased in sham animals by day 3 pi whereas that of KA-injected animals remained high throughout the experiment. These results may suggest that perikarya initiate an unconventional IF expression, which may respond to the neuronal damage induced by the mechanical injury and the excitotoxic effect of KA. It seems that vimentin and neurofilament expression may be a necessary change to promote recovery of the damaged tissue.

1. Introduction

Intermediate filaments (IF) are the major components of the cytoskeleton, together with microtubules and microfilaments. Their expression is finely tuned depending on the cell type and development [1]. In the mammalian nervous system, mainly six types of intermediate filament proteins are known to be expressed, both at central and peripheral cells [2]: (1) peripherin, a type III IF protein expressed mainly in neurons of the peripheral nervous system and neurons of the central nervous system (CNS) that have projections toward spinal motor neurons [3]; (2) vimentin, a type III IF protein expressed in mesenchymal and neuronal progenitor cells; (3) glial fibrillary acidic protein, a type III IF expressed in mature astrocytes; (4) alpha-internexin, a class IV IF, expressed in developing neuroblasts and primarily present in neurons of the adult CNS [4] (5) neurofilament, a type IV IF, which is the major component of the cytoskeleton of mature neurons [5] and, (6) nestin, a type VI IF, implicated in the radial growth of the axon expressed in mesenchymal and neuronal progenitor cells [6]. In

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http://dx.doi.org/10.1016/j.neulet.2017.02.029
0304-3940/

general, it has been shown that IF play an important role on cell and tissue integrities. Moreover, their functions are also specific to the tissue, i.e. neurofilaments involved in axonal growth and transport, dendritic arborization and changes in neuronal morphology [7–9].

Several IF expressed in the nervous system are altered in neurodegenerative diseases and cancer, and therefore, they can be used as biological markers. Perturbations of their metabolism and organization were found to be frequently associated with several neurodegenerative diseases, including Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis and giant axonal neuropathy [10,11].

Pathological features of many neurodegenerative diseases include synaptic loss, dendrite retraction and neuronal degeneration and death. It was reported that neuronal IF play a central role in damage-response mechanisms by activating a developmental program to differentiate neurons and to establish synaptic connections [12–14]. Levin et al. found the neuronal expression of vimentin in Alzheimer disease patients' brain, in Alzheimer disease transgenic mice as well as in adult mouse brains subjected to mechanical damage [12].

Kanic acid (KA) is a cyclic analog of the major stimulatory brain neurotransmitter glutamate. It acts on neuronal receptors inducing an excitotoxic effect, causing neuronal death and astrogliosis in different regions of the CNS [15] as well as on nervous cells cultures [16,17].

In a previous report, we observed behavioral and histopathological variations after intraparenchymal injection of Kainic acid (KA) in the C5 segment of the rat spinal cord [18]. Interestingly, there was a significant improvement at the motor and sensory responses as well as a mild increase in the neuronal cell count of the damaged segments by day 7 post injection (pi) of KA. In the present study, we aimed to determine whether modifications of histological characteristics in the spinal cord after KA exposure may include variations of the IF expression in neurons present at the cervical region.

2. Materials and methods

2.1. Animals

Young (3–4 mo. old, 300–400 g) ($n = 27$) male Sprague-Dawley rats, were used. Animals were housed in a temperature-controlled room (22 ± 2 °C) on a 14:10 h light/dark cycle. Food and water were available ad libitum. All experiments with animals were performed according to the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments (CICUAL) of Veterinary School Animal Welfare Assurance No. 49-8-15 P.

2.2. Toxin preparation and surgical procedure for injection

Before surgery, KA (Sigma-Aldrich, Inc., St. Louis, MO, USA) was dissolved in 0.9% saline and kept at 4 °C until use. On experimental day 0, rats were anesthetized with ketamine hydrochloride (40 mg/kg; ip) plus xylazine (8 mg/kg; im) and placed in prone position. Intraparenchymal injection of KA (1 mM) (KA-injected group) or saline (sham group) was performed as previously described [18]. Briefly, to gain access to the C5 segment trepanation at the C4–C5 fibrous joint was performed. For injecting the solution, a 10 μl Hamilton® syringe fitted with a 26 G needle was hand-held. The needle was vertically introduced 1.5 mm down on the right side of the spinal cord to reach the Lamina-VI of that side (ipsilateral). Once introduced, the needle was held in place for 2 min. Discharge of the solution lasted for 5 min. Before withdrawal, the needle was held in place for 2 more min to avoid leaking of the solution. Five μl either of the KA solution or saline were discharged at that point at a rate of 1 μl/min. Injection of KA induced a reversible loss of motor activity of the ipsilateral forelimb as previously reported [18]. Injected rats were euthanized at days 1, 3 and 7 pi. For each time point five KA-injected or three sham rats were used. One normal rat per euthanasia day was killed serving as an intact control (control group).

2.3. Specimen collection and processing

Euthanasia was performed according to the Guidelines for the Use of Animals in Neuroscience Research (the Society of Neuroscience) and the Research Laboratory Design Policy and Guidelines of NIH. Immediately before euthanasia rats were placed under general anesthesia by injection of ketamine hydrochloride (40 mg/kg, i.p.) plus xylazine (8 mg/kg; i.m.) and then intracardiacally perfused with a buffered saline-paraformaldehyde 4% solution. The vertebral column was removed and postfixed in 10% buffered formaldehyde for 24 h. The spinal cord was then dissected, immersed in cryopreservation buffer (sucrose 30%; polyvinylpyrrolidone 1%; ethylene glycol 30% phosphate buffer 1 M 1%; DW to 100 ml) and stored at −20 °C until use.

Coronal sections of C5 segment were performed under a magnifying glass as previously described [18]. Sections were serially cut into 20 μm thick coronal sections using a vibratome (Leica VT 1000S, Germany) and mounted on jellified slides. From each block, three to five sections, 120 μm apart, were analyzed.

2.4. Immunohistochemistry (IHC)

Spinal cord sections were incubated with 0.03% H_2O_2 in PBS for 30 min at room temperature. Sections were then rinsed twice in PBS and exposed to microwave antigen retrieval using a buffer citrate solution (PBS), pH 6.0. Later, sections were washed twice in PBS and incubated with 1% bovine serum albumin (BSA) in PBS for 30 min, followed by overnight incubation either with anti-neuronal nuclear antigen (NeuN, monoclonal, mouse Clone A60, Millipore, CA, USA; diluted 1:200), anti-vimentin (VIM, monoclonal, DakoCytomation, Carpinteria, CA, USA), or anti-neurofilament (NF, monoclonal, DakoCytomation,) antibody. The EnVision detection system + HRP system labeled anti-mouse or anti-rabbit polymer (DakoCytomation) was applied for 30 min. Sections were then rinsed threefold in PBS. Liquid 3,3-diaminobenzidine tetrahydrochloride (Vector Laboratories Inc., CA, USA) was used as chromogen and Hill's hematoxylin for counterstaining. Control negative sections were prepared by omitting primary antibody.

2.5. Immunofluorescence

Spinal cord sections were rehydrated with PBS containing 0.05% Tween-20 for 10 min at room temperature. Sections were exposed to microwave antigen retrieval using a buffer citrate solution (pH 6.0). Then, sections were incubated with 1% BSA in PBS for 30 min, followed by overnight incubation with neuron specific enolase (NSE, polyclonal, Sigma-Aldrich, St Louis, MO, USA), in combination either with anti-VIM or anti-NF antibodies. Sections were then rinsed threefold in PBS and incubated with 1:1000 Alexa Fluor 488-conjugated anti-mouse or Alexa Fluor 555-conjugated anti-rabbit (Invitrogen, Thermo Fisher Scientific Inc.) secondary antibody for 45 min. Then, sections were rinsed threefold in PBS and counterstained for 15 min with the fluorescent DNA stain 4′,6-diamidino-2-phenylindole. Control negative sections were prepared by omitting primary antibody.