Molecular and Cellular Neuroscience 88 (2018) 7–15

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

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

Ca^{2+} mediates axotomy-induced necrosis and apoptosis of satellite glial cells remote from the transection site in the isolated crayfish mechanoreceptor

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ARTICLE INFO

Keywords:
Axotomy
Glia
Ca^{2+}
Calcium homeostasis
Necrosis
Apoptosis

ABSTRACT

Severe nerve injury such as axotomy induces neuron degeneration and death of surrounding glial cells. Using a crayfish stretch receptor that consists of a single mechanoreceptor neuron enveloped by satellite glia, we showed that axotomy not only mechanically injures glial cells at the transection location, but also induces necrosis or apoptosis of satellite glial cells remote from the transection site. We studied Ca^{2+} role in spontaneous or axotomy-induced death of remote glial cells. Stretch receptors were isolated using the original technique that kept the neuron connected to the ventral cord ganglion (control preparations). Using Ca^{2+}-sensitive fluorescence probe fluo-4, we showed Ca^{2+} accumulation in neuronal perikarion and glial envelope. Ca^{2+} gradually accumulated in glial cells after axotomy. In saline with triple Ca^{2+} concentration the axotomy-induced apoptosis of glial cells increased, but spontaneous or axotomy-induced necrosis was unexpectedly reduced. Saline with 1/3[Ca^{2+}], oppositely, enhanced glial necrosis. Application of ionomycin, $CdCl_2$, thapsigargin, and ryanodine showed the involvement of Ca^{2+} influx through ionic channels in the plasma membrane, inhibition of endoplasmic reticulum Ca^{2+}-ATPase, and Ca^{2+} release from endoplasmic reticulum through ryanodine receptors in axotomy-induced glial necrosis. Apoptosis of glial cells surrounding axotomized neurons was promoted by ionomycin and thapsigargin. Possibly, other Ca^{2+} sources such as penetration through the plasma membrane contributed to axotomy-induced apoptosis and necrosis of remote glial cells. Thus, modulating different pathways that maintain calcium homeostasis, one can modulate axotomy-induced death of glial cells remote from the transection site.

1. Introduction

Traumatic cerebral or spinal cord injury are among main causes of people's death and disability especially in young and middle age men (Hill et al., 2016; Kobeissy, 2015). Axon transection, or axotomy, occurs not only during spine or brain trauma, but also in wounds, or during surgery. Axotomy leads either to degeneration of the damaged neuron, or to axon regeneration and restoration of its connections with other neurons, muscle fibers, and other targets. In order to treat the consequences of nerve injury, the balance between neurodegeneration and neuroprotection processes in damaged nerves should be rapidly shifted to suppression of neuronal death. Unfortunately, reliable neuroprotectors with proven efficiency are not found yet. So, deeper and comprehensive study of molecular processes that occur after axon disruption is required.

Intercellular neuroglial interactions provide the integrity of the nervous tissue and its resistance to harmful impacts. Glial cells play a considerable role in maintaining neuronal survival and regeneration after axon injury (Aldskogius and Kozlova, 1998; Giaume et al., 2013; Whiteside, 1998). Glia damage was shown to suppress the neuronal functions and induce death of neurons (Largo et al., 1996). On the other hand, nerve injury induced death of surrounding Schwann cells (Kopp et al., 1997). Localized injury of the soma in the crayfish mechanoreceptor neuron by a focused laser beam enhanced apoptosis of surrounding glial cells, thus suggesting the anti-apoptotic influence of the neuronal body on glial cells (Kolosov and Uzdensky, 2006). Nevertheless, the mechanisms of axotomy-induced lesion and death of glial cells have not been studied in detail (Verkhratsky and Butt, 2013).

Abbreviations: AT, axotomized preparation; CICR, calcium-induced calcium release; CsA, cyclosporin A; CSR, crayfish stretch receptor; MRN, mechanoreceptor neuron; Int, intact preparation; Im, ionomycin; MPTP, mitochondrial permeability transition pore; Rya, ryanodine; Tg, thapsigargin; VNC, ventral nerve cord

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https://doi.org/10.1016/j.mcn.2017.12.004
Received 21 August 2017; Received in revised form 5 November 2017; Accepted 5 December 2017
Available online 08 December 2017

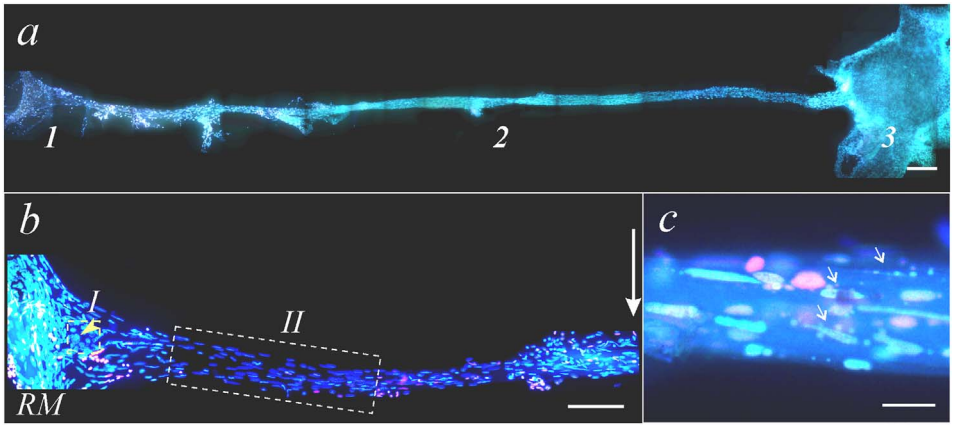

Fig. 1. Fluorescent image of the crayfish stretch receptor isolated together with its ganglion. Cell nuclei are fluorochrome-stained with Hoechst 33342 и propidium iodide (necrotic cells).
(a) The panoramic view of intact crayfish stretch receptor isolated together with corresponding ventral nerve cord ganglion. *1* – mechanoreceptor neuron body region, 2 – axon, 3 – ventral nerve cord ganglion;
(b) Axotomized stretch receptor. *RM* – receptor muscle; *I* – mechanoreceptor neuron body region; white arrowhead points out to the neuronal nucleus; *II* – proximal axon fragment; Large arrow – transection site. Dotted rectangles in *I* and *II* show the areas where necrosis and apoptosis of surrounding glial cells were estimated;
(c) Fragment of proximal axon surrounded by the nuclei of alive (blue), apoptotic (blue, fragmented), or necrotic (red) glial cells. Small white arrows on *c* indicate the fragmented nuclei of apoptotic glial cells.
Scale bars on *a* 1 mm, on *b* 500 μm, on *c* 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

As shown recently, axotomy not only mechanically injures glial cells near the cutting location, but also induces necrosis or apoptosis of satellite glial cells that are remote from the transection site (Berezhnaya et al., 2017; Khaitin et al., 2015). Various signaling pathways control survival of remote glial cells. As shown recently, MEK1/2, p38, Akt, GSK-3β and mTOR are involved in the protective, anti-apoptotic processes, whereas ERK1/2 and GSK-3β – in the anti-necrotic processes in remote glia in the axotomized crayfish stretch receptor (Berezhnaya et al., 2017).

It is unknown, how the remote glial cells get the information on axon injury. What signals induced by nerve transection propagate to remote glial cells and cause their death? One possible molecular signal that can propagate from the transected nerve site to remote glial cells is Ca^{2+}. Ca^{2+} is a key element of many signaling pathways, which control the physiological state of the cell. Calcium ions play an important role in neurodegeneration (Gemes et al., 2009; Marambaud et al., 2009), in particular, in neuronal responses to axotomy (Hilaire et al., 2005; Kobeissy, 2015; Rishal and Fainzilber, 2014; Sánchez-Vives et al., 1994; Siedler et al., 2014). Elevation of the cytosolic Ca^{2+} level to 10^{-4}–10^{-3} M can trigger cell death, necrosis or apoptosis (Berliocchi et al., 2005; Kondratskyi et al., 2015; Zhivotovsky and Orrenius, 2011). The exact role of Ca^{2+} in survival and death of remote glial cells after axotomy is still unclear. It is unknown, which mechanisms that maintain Ca^{2+} homeostasis, regulate survival of glial cells that surround the axotomized neuron.

The abdominal crayfish stretch receptor (CSR) is a simple but informative model object for studying the neuronal and glial responses to axotomy and neuroglial interactions. It contains the single bipolar mechanoreceptor neuron (MRN) mounted on the receptor muscle that is stretched between the adjacent abdominal segments. Its axon is directed to the ventral cord ganglion. MRN is surrounded by the multilayer roll-like glial envelope (Fedorenko and Uzdensky, 2009). The advantage of CSR is that both MRN and satellite glial cells, which envelope namely this neuron, are well identified at the optical microscopy level. The ultrastructure of MRN and satellite glial cells, neuron-glia interactions, signaling mechanisms, which control necrosis and apoptosis of these cells under various physical and chemical impacts have been described previously in detail (Fedorenko and Uzdensky, 2009; Fedorenko et al., 2011; Uzdensky, 2010; Uzdensky et al., 2015). In the course of CSR isolation, MRN axon is usually transected. Transected neurons survive and fire 8–10 h or more after isolation with a frequency almost proportional to the receptor muscle extension (Rydqvist et al., 2007). The novel technique of CSR isolation without transection of MRN axon, which preserves the neuron connection to the ventral nerve cord ganglion, have been recently developed (Khaitin et al., 2015). Such undamaged (intact) CSR preparations were used as control ones in the present work.

Here we have studied the role of Ca^{2+} in the axotomy-induced death of glial cells that surround the proximal MRN segment, which is remoted from the transection site. The dynamics of Ca^{2+} accumulation in the glial envelope was studied. The application of different inhibitors revealed the roles of various pathways that maintain the calcium homeostasis in remote glial cells after axon transection: Ca^{2+} channels, Ca^{2+}-ATPase of endoplasmic reticulum (SERCA), ER ryanodine receptors, and mitochondrial permeability transition pores (MPTP).

2. Materials and methods

2.1. Chemicals

The Ca^{2+} fluorescence probe Fluo-4 (Life Technologies, USA) was dissolved in pluronic F-127 (Invitrogen, USA). Calcium ionophore ionomycin, inhibitor of ER ryanodine receptors ryanodine, and SERCA inhibitor thapsigargin were obtained from Alomone Labs Ltd. (Israel). MPTP inhibitor cyclosporin A and fluorochromes propidium iodide and Hoechst 33342 were obtained from Sigma-Aldrich-Rus (Moscow, Russia).

2.2. Crayfish stretch receptor preparation

The crayfishes *Astacus leptodactylus* from the Don River affluences were purchased from the local market. The abdominal crayfish stretch receptor was isolated without MRN transection according to the recently developed technique (Khaitin et al., 2015), so that its axon remains connected with the ventral nerve cord ganglion (Fig. 1a). After isolation, CSR was incubated 8 h (time necessary for apoptosis development) at room temperature (22–25 °C) in dark in a 2 ml plexiglass chamber filled with van Harreveld's saline (mM: NaCl - 205; KCl - 5.4; $NaHCO_3$ - 0.24; $CaCl_2$ - 13.5; $MgCl_2$ - 5.4; pH 7.2–7.4) (Van Harreveld, 1936) in the presence or absence of calcium homeostasis modulators. To assure that MRN is alive and physiologically active after isolation, its spikes were derived extracellularly from axon by a glass pipette suction electrode, amplified, digitized, and processed on a personal computer using the home-made software, which provided continuous monitoring and recording of firing frequency. Such intact preparations (Int) were used as control. To prepare the axotomized preparations (AT) we first isolated the intact MRN as for control, registered its firing, and then transected the axon with sharp ophthalmic scissors at a distance of about 8–10 mm from the neuronal body (Fig. 1b). The initial firing frequency was preliminary established at 6 to 10 Hz by stretching of the receptor muscle. The methods of electron-microscopic study of MRN have been described earlier (Fedorenko and Uzdensky, 2009).