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Research Report

Hippocampal GABAergic interneurons coexpressing alpha7-nicotinic receptors and connexin-36 are able to improve neuronal viability under oxygen–glucose deprivation

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ABSTRACT

The hippocampal interneurons are very diverse by chemical profiles and rather inconsistent by sensitivity to CI. Some hippocampal GABAergic interneurons survive certain time after ischemia while ischemia-sensitive interneurons and pyramidal neurons are damaged. GABAergic signaling, nicotinic receptors expressing α7-subunit (α7nAChRs$^+$) and connexin-36 (Cx36$^+$, electrotonic gapjunctions protein) contradictory modulate post-ischemic environment. We hypothesized that hippocampal ischemia-resistant GABAergic interneurons coexpressing glutamate decarboxylase-67 isoform (GAD67$^+$), α7nAChRs$^+$, Cx36$^+$ are able to enhance neuronal viability. To check this hypothesis the histochemical and electrophysiological investigations have been performed using rat hippocampal organotypic culture in the condition of 30-min oxygen–glucose deprivation (OGD). Post-OGD reoxygenation (4 h) revealed in CA1 pyramidal layer numerous damaged cells, decreased population spike amplitude and increased pair-pulse depression. In these conditions GAD67$^+$ interneurons displayed the OGD-resistance and significant increase of GABA synthesis/metabolism (GAD67-immunofluorescence, mitochondrial activity). The α7nAChRs$^+$ and Cx36$^+$ co-localizations were revealed in resistant GAD67$^+$ interneurons. Under OGD: GABAA-receptors (GABAARs) blockade increased cell damage and exacerbated the pair-pulse depression in CA1 pyramidal layer; α7nAChRs and Cx36-channels separate blockades sufficiently decreased cell damage while interneuronal GAD67-immunofluorescence and mitochondrial activity were similar to the control. Thus, hippocampal GABAergic interneurons co-expressing α7nAChRs and Cx36 remained resistant certain time

Abbreviations: α7nAChRs, α7 nicotinic acetylcholine receptors; ACSF, artificial cerebrospinal fluid medium; CBX, carbenoxolone; CI, cerebral ischemia; CM, culture medium; Cx36, connexin36; GABA, γ-aminobutyric acid; GAD67, glutamic acid decarboxylase-67 isoform; GBZ, gabazine; ISI, inter-stimuli interval; MFQ, mefloquine; MLA, methyllycaconitine; MTO, MitoTracker Orange; NMDA, N-methyl-D-aspartate; OGD, oxygen–glucose deprivation; PBS, phosphate buffer saline; PFA, paraformaldehyde; PI, propidium iodide; PPD, paired pulse depression; PS, population spikes

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after OGD and were able to modulate CA1 neuron survival through GABAARs, α7nAChRs and Cx36-channels activity. The enhancements of the neuronal viability together with GABA synthesis/metabolism normalization suggest cooperative neuroprotective mechanism that could be used for increase in efficiency of therapeutic strategies against post-ischemic pathology.

1. Introduction

Hippocampal interneurons are very diverse in their sensitivity and vulnerability to the cerebral ischemia (CI) (Francis and Pulsinelli, 1982; Nitsch et al., 1989; Johansen, 1993; Schwartz-Bloom and Sah, 2001). Some GABAergic interneurons keep their functionality certain time after the stroke, while ischemia-sensitive interneurons and pyramidal neurons start dying shortly after the insult (Pulsinelli and Brierley, 1979; Shinno et al., 1997).

Neuronal death caused by CI both in vivo and in vitro is thought to be due to glutamate-induced neurotoxicity (Olney and Sharpe, 1969; Choi, 1987) and toxic Ca^{2+} overload (Choi, 1995; Mark et al., 2001; Harukuni and Bhardwaj, 2006; Marks, 2009). Beside NMDA receptors, hippocampal Ca^{2+} overloads could be mediated by the nicotinic receptors expressing the α7-subunit (α7nAChRs) (Castro and Albuquerque, 1995; Uteshev, 2012) and by connexin-36 (Cx36) constituting gap-junctions between GABAergic interneurons (Hormuzdi et al., 2001; Oguro et al., 2001; Thompson et al., 2006; Wellershaus et al., 2008; Allen et al., 2011; Park et al., 2011). CI induces also sufficient increase of the GABA synthesis/release and extracellular accumulation, $GABA_A$ receptors ($GABA_A$Rs) and Cl^- gradient alterations (Rothman, 1985; Choi, 1987; Schwartz-Bloom and Sah, 2001; Allen et al., 2004).

GABAergic signaling, α7nAChRs, Cx36-channels can be either neuroprotective or degenerative, depending on CI-affected environment (Oguro et al., 2001; Kawai et al., 2002; Ferchmin et al., 2003; Allen et al.; 2004; Park et al., 2011; Uteshev, 2012). Consequently, pharmacologic interventions separately normalizing hippocampal GABA-neurotransmission, α7nAChRs, or Cx36-channels are not sufficient to restore neurological functions after a stroke (Johansen, 1993; Allen et al., 2004; Zhan et al., 2007). In some measure, this problem is due to the incomplete data on the cell-specific expression and cooperation of GABA-mechanisms, α7nAChRs and Cx36 in hippocampus under CI-conditions (Oguro et al., 2001; Kawai et al., 2002; Wellershaus et al., 2008; Son et al., 2008; Park et al., 2011).

In present study we examined the rat hippocampal organotypic culture 1 and 4 h after 30-min oxygen–glucose deprivation (OGD) for two aims: whether OGD-resistant neurons coexpress the glutamate decarboxylase-67 isoform (GAD67), α7nAChRs and Cx36 and whether separate blockades of $GABA_A$Rs, α7nAChRs and Cx36 gap-junctions modulate the neuronal survival under OGD. We demonstrate that hippocampal interneurons immunopositive for GAD67, α7nAChRs and Cx36 are temporary OGD-resistant and are able to modulate the neuronal viability in CA1 pyramidal layer with an obvious impact of $GABA_A$Rs, α7nAChRs and Cx36. Enhanced neuronal survival and GABA synthesis/metabolism normalization under α7nAChRs and Cx36-channels blockades suggest an intracellular cooperative mechanism that is important for neuronal post-OGD survival. The interplay of the GABA-, α7nAChRs- and Cx36-signaling systems could be used to increase in efficiency of therapeutic strategies against the post-ischemic pathology.

Table 1 – The effects of separate blockades of the $GABA_A$Rs, α7nAChRs and Cx36-channels on the number of OGD-damaged cells in CA1 pyramidal layer.

Control	Control	OGD+1 h	OGD+4 h
	2.62±0.36, n=29		
OGD		8.50±1.01, n=42	31.32±2.65, n=31
GABAARs (GBZ)	6.07±1.3, n=14	13.17±1.5, n=23	35.26±2.77, n=19
A7nAChRs (MLA)	12.13±1.42, n=23	6.43±1.02, n=7	29.08±3.89, n=14
Cx36 (CBX)	7.89±1.33, n=9	5.67±0.22, n=12	12.93±2.11, n=28
Cx36 (MFQ)	7.01±1.2, n=5	4.14±0.59, n=7	17.86±3.25, n=7

In each section: Mean±SEM of the $PI^+/GAD67^-$ cells per mm^2, n—the number of counts.
Abbreviations:
Control—normal culture without OGD and blocker applications.
$GABA_A$Rs (GBZ)—the $GABA_A$-receptors blockade with 10 μM gabazine.
α7nAChR (MLA)—the nicotinic acetylcholine receptors expressing α7-subunits blockade with 5 μM methyllicaconitine.
Cx36 (CBX) or Cx36 (MFQ)—the connexin-36 expressing gap-junctions blockade with 75 μM carbenoxolone or 300 nM mefloquine, correspondently.
OGD+1 h—a culture under 30-min OGD followed with 1 h in normal culture medium (CM).
OGD+4 h—a culture under30-min OGD followed with 4 h in CM.