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Research report

Epigenetic regulation of death of crayfish glial cells but not neurons induced by photodynamic impact

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ABSTRACT

Epigenetic processes are involved in regulation of cell functions and survival, but their role in responses of neurons and glial cells to oxidative injury is insufficiently explored. Here, we studied the role of DNA methylation and histone deacetylation in reactions of neurons and surrounding glial cells to photodynamic treatment that induces oxidative stress and cell death. Isolated crayfish stretch receptor consisting of a single mechanoreceptor neuron surrounded by glial cells was photosensitized with aluminum phthalocyanine Photosens that induced neuron inactivation, necrosis of the neuron and glia, and glial apoptosis. Inhibitors of DNA methylation 5-azacytidine and 5-aza-2′-deoxycytidine (decitabine) reduced the level of PDT-induced necrosis of glial cells but not neurons by 1.3 and 2.0 times, respectively, and did not significantly influence apoptosis of glial cells. Histone deacetylase inhibitors valproic acid and trichostatin A inhibited PDT-induced both necrosis and apoptosis of satellite glial cells but not neurons by 1.6–2.7 times. Thus, in the crayfish stretch receptor DNA methylation and histone deacetylation are involved in epigenetic control of glial but not neuronal necrosis. Histone deacetylation also participates in glial apoptosis.

1. Introduction

Photodynamic therapy (PDT) is based on photoinduced generation of strongly cytotoxic singlet oxygen, following oxidative stress and death of stained cells under light exposure in the presence of oxygen. It is currently used in oncology (Agostinis et al., 2011) including treatment of brain tumors (Kostron, 2010; Madsen et al., 2006). However, in the last case not only malignant, but also surrounding normal neurons and glial cells are damaged that can induce unacceptable side effects and neurological disorders. Therefore, PDT effect on the normal nervous tissue should be carefully investigated.

Cell reactions to various impacts including PDT and their death are controlled by the complex signaling system that consists of thousands proteins that form the intracellular regulatory network (Buytaert et al., 2007; Gomperts et al., 2009; Uzdensky, 2008). If the regulatory potential of the proteins present in the cell is insufficient, additional protein synthesis is stimulated. Gene expression is controlled by transcription factors and epigenetic regulators: DNA methylation/demethylation and histone modifications such as methylation/demethylation, acetylation/deacetylation, and phosphorylation/dephosphorylation, which regulate access of transcription factors and RNA polymerase II to gene promoters. Aberrant DNA methylation and histone modifications are involved in diverse neuronal functions and neurological disorders such as synaptic plasticity and memory formation (Sultan and Day, 2011), Alzheimer, Parkinson, and Huntington diseases (Gebicke-Haerter, 2012; Gray, 2011; Zawia et al., 2009), schizophrenia (Harrison and Dexter, 2013), epilepsy (Hwang et al., 2013), acute and chronic stress (Stankiewicz et al., 2013), and stroke (Hwang et al., 2013). However, there are very few direct data on the involvement of epigenetic processes in neurodegeneration (Chestnut et al., 2011).

The role of epigenetic processes in the reactions of the normal nervous tissue to photodynamic treatment remains almost unstudied. Using the proteomic approach we have recently shown that PDT influences the expression of various proteins involved in epigenetic regulation in the murine cerebral cortex such as histone deacetylases HDAC-1 and HDAC-11, transcriptional repressors Kaiso and dimethylated histone H3, transcription factors AP-1/c-Jun and FOXC2, phosphorylated histone H2AX involved in DNA repair, importin α5/7, protein methyltransferase PRMT5, and some others (Demyanenko et al., 2013). However, the study

Abbreviations: DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HDAC, histone deacetylase; PDT, photodynamic therapy.

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of the complex brain tissue that consists of different cell types including neurons, glial cells, and blood vessels does not provide the information on epigenetic regulation in different cell types.

The suitable object for study of epigenetic processes occurring simultaneously in the interacting neuronal and glial cells is the crayfish stretch receptor that consists of a single mechanoreceptor neuron surrounded by glial cells, which form a multilayer envelope around the neuron (Fedorenko and Uzdensky, 2009). The role of diverse signaling and metabolic processes in responses of crayfish neurons and glial cells to photodynamic treatment has been previously studied (Komandirov et al., 2011; Kovaleva et al., 2013; Uzdensky et al., 2005, 2007, 2013).

In the present work we studied the role of DNA methylation and histone deacetylation in PDT-induced inactivation and death of the stretch reception neuron and surrounding glial cells. We showed that inhibitors of DNA methylation 5-azacytidine and 5-aza-2′-deoxycytidine (decitabine) inhibited PDT-induced necrosis of glial cells but not neurons, whereas inhibitors of histone deacetylase (HDAC) valproic acid and trichostatin A inhibited PDT-induced necrosis and apoptosis of satellite glial cells but not neurons.

2. Materials and methods

2.1. Chemicals

The following chemicals were used: DNA methyltransferase inhibitors 5-azacytidine (5-Aza, 10 μM) and 5-aza-2′-deoxycytidine (decitabine, 10 μM); histone deacetylase inhibitors valproic acid, sodium salt (VPA, 0.5 mM) and trichostatin A (TSA, 100 nM); fluorochromes propidium iodide and Hoechst 33342. All chemicals were obtained from Sigma–Aldrich-Rus (Moscow, Russia). Photosensitizer Photosens, a mixture of sulphonated aluminum phthalocyanines, $AlPcS_n$, where mean $n = 3.1$, was obtained from NIOPIK (Moscow, Russia).

2.2. Crayfish stretch receptor and recording of its firing

The crayfishes *Astacus leptodactylus* from Don River affluences were purchased on the local market. Their abdominal stretch receptors were isolated as described by Florey and Florey (1955). These were placed into a plexiglass chamber equipped with a device for receptor muscle extension and filled with 2 ml of van Harreveld saline (mM: NaCl – 205; KCl – 5.4; $NaHCO_3$ – 0.2; $CaCl_2$ – 13.5; $MgCl_2$ – 5.4; pH 7.2–7.4). Neuron spikes were recorded extracellularly from axons by glass pipette suction electrodes, amplified, digitized by the analog-digital converter L-761 (L-Card, Moscow, Russia), and processed by a personal computer using the home-made software that provided continuous monitoring of firing. Experiments were carried out at 23 ± 4 °C.

2.3. Photodynamic treatment and application of enzyme inhibitors

At the beginning of the experiment the initial level of neuronal activity was set near 6–10 Hz by application of the appropriate receptor muscle extension. After 30 min control recording of neuronal activity, Photosens (75 nM) and an inhibitor were added into the chamber with an interval of 3–5 min. After following 30-min incubation, cells were irradiated with the He–Ne laser (633 nm, 0.3 W/cm^2). A laser beam diameter was of 3 mm, so that the neuronal body and a significant part of axon were irradiated. 30-min light exposure was longer than the duration of bioelectric neuron response measured from the irradiation start to the moment of firing abolition (typically, 10–20 min), which we called the “neuron lifetime”. Photosens and an inhibitor were present in the chamber during and after irradiation. The inhibitor concentration was usually chosen to be approximately 2 times lower than the predetermined concentration, which disturbed neuron firing in the darkness for 3–4 h.

2.4. Cell death assay

In order to visualize dead neurons and glial cells, 20 μM propidium iodide and 10–20 μM Hoechst 33342 (both from Sigma–Aldrich) were added into the experimental chamber at 8 h postirradiation. This time interval was sufficient for apoptosis development (Uzdensky et al., 2005). Then preparations were washed with van Harreveld saline, fixed with 0.2% glutaraldehyde, repeatedly washed and mounted in glycerol. Fluorescent images were acquired using the fluorescence microscope Lumam-I3 (LOMO, Sankt-Petersburg, Russia) equipped with a digital photocamera. Propidium iodide, a membrane impermeable fluorochrome, imparts red fluorescence to nuclei of necrotic cells with the compromised plasma membrane. Hoechst 33342 imparts blue fluorescence to the nuclear chromatin. It visualizes intact nuclei of living cells and fragmented nuclei of apoptotic cells (Fig. 1). Nucleus fragmentation is the final stage of apoptosis when the no-return point has passed. It should be mentioned that other methods for apoptosis evaluation such as caspase activation, cytochrome c release, or annexin V assay, which require observation of the cytoplasm or of the plasma membrane, are not suitable for study of glial cells in the isolated stretch receptor because of their multilayer roulette-like morphology and overlapping of optical images of different glial processes and the neuronal cytoplasm (Fedorenko and Uzdensky, 2009). The red nuclei of necrotic glial cells stained by propidium iodide were counted in the predetermined standard field (100 μm × 100 μm) around SRN soma so that the neuron nucleus was situated in its center. Fragmented nuclei of apoptotic glial cells were counted around the proximal 2 mm axon fragment where glial apoptosis was more profound than around the neuron body. Their mean number representing the level of glial apoptosis was expressed below as relative units. The one way ANOVA was used for statistical evaluation of the difference between independent experimental groups. Data are presented as mean ± S.E.M.

3. Results

Neither Photosens (75 nM), nor laser radiation separately changed significantly neuronal activity and survival after stretch receptor isolation. However, their combined action, i.e. PDT led to inhibition of neuronal activity, necrosis of neurons and glial cells and apoptosis of glial cells (Figs. 2–5). Apoptotic nuclear fragmentation was never observed in neurons as described earlier (Uzdensky et al., 2002, 2005).

In the darkness, both inhibitors of DNA methyltransferase 5-azacytidine (10 μM) and decitabine (10 μM) did not influence significantly the duration of bioelectric neuron response (Figs. 2A and 3A) and the level of neuronal necrosis (Figs. 2B and 3B). However, 5-azacytidine more than 2 times increased the level of glial necrosis ($p < 0.05$; Fig. 2C) and showed the similar tendency for glial apoptosis ($p > 0.05$; Fig. 2D). Unlike, decitabine did not demonstrate glia toxicity in the darkness (Fig. 3C and D).

5-Azacytidine and decitabine did not influence the PDT-induced changes in the neuronal activity and necrosis level (Figs. 2A and B, 3A and B). We also did not observe significant effects of these DNA methyltransferase inhibitors on PDT-induced glial apoptosis (Figs. 2D and 3D). However, both of them reduced the levels PDT-induced necrosis of glial cells by 1.3 and 2.0 times ($p < 0.05$; Fig. 2C and $p < 0.01$; Fig. 3C, respectively).