Laser-Induced Singlet Oxygen Stimuates Bioenergetics of Insulin-Producing Cells

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The paper presents the results of the study of the effect of singlet oxygen induced by 1267 nm laser irradiation without the use of photosensitizers on the bioenergetics of rat RINm5F insulinoma cells.

Key words: pancreatic β-cells, RINm5F insulinoma cells, reactive oxygen species, singlet oxygen, mitochondrial membrane potential, mitochondrial transition pore permeability, NADH

I. Introduction

Today, diabetes mellitus (DM) affects one in 11 people worldwide, so the search for strategies for early treatment of DM is an urgent public health issue. One such approach being developed is to restore the function of pancreatic β-cells responsible for insulin secretion.

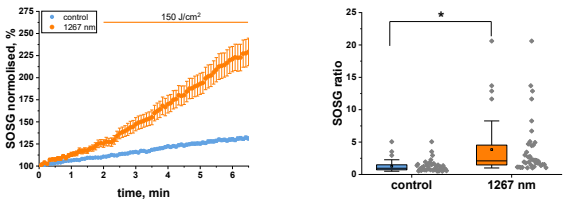
Adenosine triphosphate (ATP) production by β-cell mitochondria, which is impaired in DM, is a major factor in insulin secretion [1]. Therefore, it seems promising to consider the activation of mitochondrial respiration and ATP production by mitochondria of β-cells as a therapeutic strategy of DM. It has recently been demonstrated that direct laser generation of singlet oxygen (SO) can serve as an activator of mitochondrial respiration and ATP production [2]. This work served as the basis for our research.

II. Material and Methods

Studies were carried out on rat insulinoma RINm5F cells (a model of pancreatic islet beta cells) with 40-50% confluence by confocal microscopy (mitochondrial membrane potential and SO production) and fluorescence microscopy (NADH autofluorescence). SO generation was carried out using a device that was developed, including a laser radiation source with a wavelength of 1267 nm to excite the oxygen molecules.

III. Experimental Results and Discussion

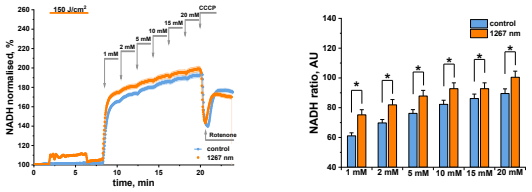
Using a fluorescent SOSG probe (singlet oxygen sensor green), characterized by high selectivity to SO, it was found that 1267 nm laser irradiation activates SO production in insulinoma cells, which allows us to apply this approach in further studies (Fig. 1).



a) b)

Fig.1. Normalized averaged probe fluorescence signal with and without the 1267 nm laser (a) and fluorescence intensity increases (b).

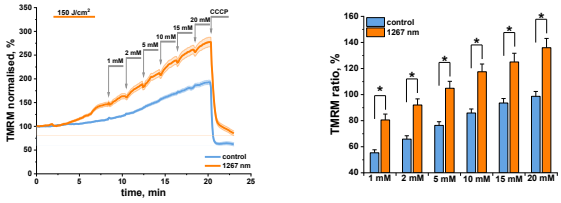
SO generated by the 1267 nm laser resulted in a greater increase in NADH autofluorescence indicating increase of NAD reduction after each addition of glucose to cells in glucose-free HBSS compared to the experiment without the laser (Fig.2).



a) b)

Fig. 2. Normalized averaged NADH autofluorescence signal with and without 1267 nm laser (a) and increases in NADH autofluorescence intensity with glucose addition supplementation with and without the 1267 nm laser exposure (b).

As can be seen, the mitochondrial membrane potential when glucose is added is higher when SO is generated by 1267 nm laser irradiation than when glucose is added to non-irradiated cells (Fig. 3).



a) b)

Fig. 3. Normalized averaged signal of the mitochondrial membrane potential with and without 1267 nm laser (a) and signal increases with glucose supplementation with and without the 1267 nm laser exposure (b).

Differences in changes of analyzed parameters of the investigated cells after laser treatment were revealed in comparison with the control group of cells that were not exposed to laser 1267 nm.

The results obtained at this stage indicate a potential possibility of using laser-induced SO in the regulation of β-cell functions.

Acknowledgment

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References

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