ISSN 1607-6729, Doklady Biochemistry and Biophysics, 2014, Vol. 457, pp. 146–148. © Pleiades Publishing, Ltd., 2014.
Original Russian Text © A.L. Drozdov, T.I. Karu, V.M. Chudnovskii, V.I. Yusupov, V.N. Bagratashvili, 2014, published in Doklady Akademii Nauk, 2014, Vol. 457, No. 4, pp. 487–490.

BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

Influence of Low-Intensity Red Diode and Laser Radiation on the Locomotor Activity of Sea Urchin Sperm

A. L. Drozdov[b, d], T. I. Karu[a], V. M. Chudnovskii[c, d], V. I. Yusupov[a, c], and V. N. Bagratashvili[a]

Presented by Academician V.Ya. Panchenko October 7, 2013

Received April 9, 2014

DOI: 10.1134/S1607672914040085

Monochromatic (laser) and quasimonochromatic light in the range 600–900 nm can stimulate metabolic processes in eukaryotes [1–4]. Despite the fact that this phenomenon has already been successfully used for decades in medical practice (low-intensity laser therapy), the range of possible applications of this type of radiation is still expanding, and the cellular mechanisms of interaction of the laser beam with cells are investigated.

An increase in the motor activity of sperms in order to improve their quality is an important component for any fertility treatment and artificial insemination [3]. It is known that, for successful fertilization, the sperm must dramatically increase the velocity in the shortest time [5], which requires activation of mitochondrial energy production. Earlier, it was shown that irradiation of sea urchin sperms with a helium–neon laser increases their lifespan [6]. The aim of our experiments was to study the possibilities of increasing the motor activity of sperms exposed to low-intensity laser (635 nm) and quasimonochromatic (650 ± 10 nm) radiation. It is known that exposure to light in the red and far-red regions of the spectrum can accelerate the functioning of mitochondria after the absorption of light by the respiratory chain enzyme cytochrome c oxidase [7]. The effects of laser biostimulation are the consequence of electronic excitation of chromophores Cu_A and Cu_B in the molecule of this enzyme. The change in the degree of oxidation of cytochrome c oxidase and accelerated transfer of electrons to the catalytic site of the enzyme are considered as the main mechanism of action of biostimulation [7].

MATERIALS AND METHODS

The study was performed at the Vostok biological station, Institute of Marine Biology, Far East Branch, Russian Academy of Sciences. The study object was the sperms of the gray sea urchin *Strongylocentrotus intermedius* (Strongylocentrotidae, Echinoidea, Echinodermata), which are the classical model for studying the mechanisms of sperm movement [5]. Sea urchins were collected at a depth of 3–5 m at a temperature of 17–20°C and salinity of 31–32‰. The urchins were housed in baths filled with purified sea water at a temperature of 20°C. All further studies were performed in a special darkened and air-conditioned box at a temperature of 20°C. Gametes for experiments were obtained by injecting approximately 1 mL of 1 M KCl into the shell cavity of animals ready to spawn. Gametes were released from the gonopores located on the aboral side of urchins (eggs from females and sperms from males) 1–3 min after the injection and collected to a jar with sea water. The sperm concentration in the jar was approximately 10^5–10^7 pcs. /mL. The sperm suspension (6 mL) was placed in 200-mL beakers and in Petri dishes 33 mm in diameter. In the upper third of the beakers, the sperms retained motility for 36 h; in Petri dishes, for approximately 20 h. The motility of sperms was studied under a Leica DM2500 microscope (Germany) equipped with a digital camera (Digital B/W camera RC-1002E, Taiwan) performing shooting at 25 frames/s. Immediately before analysis, an aliquot of sperm suspension (approximately 0.2 mL) was taken from the Petri dish with a pipette, placed on a glass slide in a well 15 mm in diameter, and covered with a cover glass 150 μm thick, so that there remained no bubbles under the glass.

The Petri dishes with sperm were irradiated with monochromatic (laser) and broadband quasimonochromatic (LED) light of the red wavelength range. A

[a] *Institute of Laser and Information Technologies, Russian Academy of Sciences, Troitsk, Moscow oblast, 142190 Russia*
[b] *Zhirmunskii Institute of Marine Biology, Far East Branch, Russian Academy of Sciences, ul. Pal'chevskogo 17, Vladivostok, 690059 Russia*
[c] *Il'ichev Pacific Oceanological Institute, Far East Branch, Russian Academy of Sciences, Vladivostok, 690041 Russia; e-mail: iouss@yandex.ru*
[d] *Far Eastern Federal University, Vladivostok, 690091 Russia*

laboratory device with a red LED matrix, with radiation in the region of 650 nm and a spectral width of 20 nm was used as a quasimonochromatic light source. The matrix 12 cm in diameter containing 19 LEDs was placed on a tripod at an elevation of 10 cm from the table. The broadband red light intensities at the table level were 90, 250, and 750 mW/cm^2, and the exposure doses were 7×10^{-5}, 7×10^{-4}, 7×10^{-3}, and 7×10^{-2} J/cm^2. A laboratory device based on a semiconductor laser diode emitting in the region of 635 nm with a spectral width of 2 nm, which was placed on a tripod at an elevation of 12.5 cm from the surface of the table, was used as a source of laser light. Petri dishes were irradiated with a laser light intensity of 290 mW/cm^2 and exposure dose of 3×10^{-3} J/cm^2. An unexposed sperm suspension in a Petri dish served as a control.

To control the spectral composition and intensity of laser radiation, we used an USB 4000 optic spectrum analyzer (Ocean Optics, United States) connected with a PC, with a wavelengths range from 200 to 1100 nm, and a FieldMaster power meter with an LM-10HTD measuring head (Coherent, United States). Statistical processing of the quantitative results was performed using the Student's t test, counting the arithmetic mean and the standard deviation. Differences were considered statistically significant at $p \le 0.05$.

RESULTS AND DISCUSSION

After getting from the male gonoducts to seawater, mature sperms begin to move continuously and chaotically. Gradually, their movement is slowed down, and many of them completely lose their mobility. One day later, the number of motile sperms in the Petri dish becomes smaller than at the beginning of the experiment, when 100% of sperms were motile. In addition, the nature of sperm movement changes. Their motions become discontinuous: they may stay immobile for some time, then move for a few seconds, stop again, and then become motile again.

To assess the effect of low-intensity radiation on sperms, we used a new technique developed by us for isolation of active sperms, which is based on determination of the activity factor. Observations of sea urchin sperms that could be well distinguished during visual examination under a microscope (lens 20×, 40×, and 100×) and on micrographs showed that they move highly unevenly (Fig. 1): the periods of rapid movements are alternated with the periods of slow movements and even almost complete immobility. Figure 1 shows the changes in the movement velocity over time using two sperms as an example: more active (1) and less active (2). As can be seen from the figure, most of the time both sperms moved at low velocities (0–30 μm/s). Their maximum velocities in the periods of rapid movement significantly differed and were approximately 600 and 50 μm/s for the more active (1) and less active (2) sperms, respectively. At the same time,

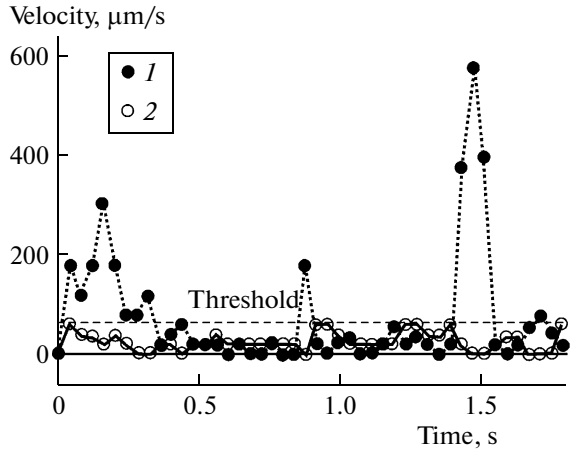

Fig. 1. Typical example of changes in velocity over time of (*1*) more active and (*2*) less active sperms.

the sperm suspensions almost always contained the sperms that remained stationary for the time of observation (approximately 1 min).

The factor showing the percentage of active sperms that shifted from their place at a distance greater than 2.5 μm (about half the length of the sperm) during the time elapsed between two successive frames (40 ms) was selected as a criterion of sperm activity. The velocity of such active sperms must exceed the threshold of 62 μm/s (dashed line in Fig. 1). As can be seen in Fig. 1, the velocities of the less active sperm (curve *2*) during the entire observation period did not exceed the set threshold values. The more active sperm was moving faster than the velocity threshold for about 25% of this time. To determine the activity factor using a computer program (MATLAB package), we viewed the films frame by frame. IN this case, the algorithm of this program identified those sperms that shifted from their place in the previous frame by a distance more than 2.5 μm and determined the percentage of such sperms. Thus, for each measurement, we performed computer analysis of 25 consecutive frames and calculated the activity factor as the mean (over a period of 1 s) percentage of active sperms. In total, we performed three independent measurements ($n = 3$), on the basis of which the mean value and the standard deviation were determined.

As can be seen in Fig. 2, the activity factor of sperms significantly differed from the control already 15 min after a short-term exposure. It should be noted that, although the exposure to relatively high doses of the broadband light significantly (by 70–90%) increased the activity factor, the lowest doses did not cause its significant changes.

As can be seen in Fig. 3, 105 min after exposure to the broadband red light, a significant increase in the activity factor in the middle of the selected range and a nonsignificant decrease at the highest dose selected were observed. Note that such an effect of the dose of

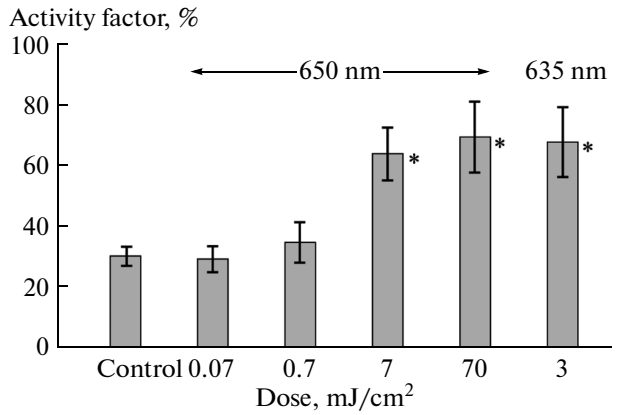

Fig. 2. Sperm activity factor 15 min after the irradiation with light of different wavelengths and doses. Here and in Fig. 3, the asterisk marks the results that significantly differed from the control ($p < 0.05$, $n = 3$).

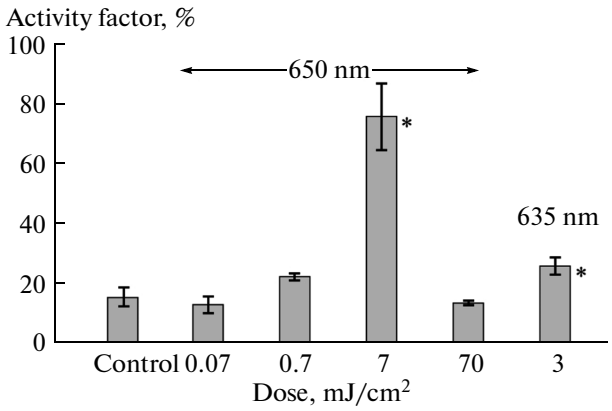

Fig. 3. Sperm activity factor 105 min after the irradiation with light of different wavelengths and doses.

7 mJ/cm^2 significantly (5 times) increased the activity factor. As for the effect of the used doses of the narrow-band laser radiation on the sperm motility, one can see that irradiation in this case resulted in a significant increase in the activity factor relative to the control—by almost 100% 15 min after exposure (Fig. 2) and 60% 105 min after exposure (Fig. 3).

The results of our experiments showed that, in a short time after irradiation (15 min), the activity factor increased in dose-dependent manner: relatively high doses (3, 7, and 70 mJ/cm^2) caused an almost twofold increased in the sperm activity (Fig. 2). At a longer time (105 min), the factor of activity of cells in the irradiated samples remained practically at the control level, except the dose of 7 mJ/cm^2, at which it approximately 5 times exceeded the control values (Fig. 3).

Our data confirm the idea that irradiation of cells with light in the red region of the spectrum can accelerate the function of the mitochondrial apparatus. Sea urchin sperm is arranged very simply: this is a flagellated cell with a conical nucleus, approximately 4 μm in length and approximately 1 μm in diameter at the base. At the base of the nucleus, there is one circular mitochondrion, in the lumen of which centrioles are located, one of which functions as a kinetosome of the flagellum [8]. Of all the components of the sperm, only the components of the mitochondrial respiratory chain are sensitive to red light radiation [7]. The effects of laser biostimulation are the consequence of electronic excitation of chromophores Cu_A and Cu_B in this molecule. The change in the degree of oxidation of cytochrome c oxidase and accelerated electron transfer to the catalytic site of the enzyme are considered as the main mechanism of action of biostimulation [9].

In this study, we showed that low-intensity red light diode and laser radiation affect the locomotor activity of sea urchin sperms, 2–5 times increasing the percentage of active cells depending on the time elapsed after exposure.

ACKNOWLEDGMENTS

The study was supported by the Russian Foundation for Basic Research (project nos. 12-02-00392, 10-02-00672, 12-02-10013, and 13-02-10013) and the Russian Ministry of Sciences and Education (agreement no. 8144).

REFERENCES

1. Karu, T., *IEEE J. Quantum Electron.*, 1987, QE-23, no. 10, pp. 1703–1717.
2. Vladimirov, Yu.A., Osipov, A.N., and Klebanov, G.I., *Biokhimiya*, 2004, vol. 69, no. 1, pp. 103–113.
3. Karu, T.I., *Photomed. Laser Surg.*, 2012, vol. 30, no. 5c, pp. 249–251.
4. Chudnovskii, V.M., Leonova, G.N., Skopinov, S.A., Drozdov, A.L., and Yusupov, V.I., *Biologicheskie modeli i fizicheskie mekhanizmy lazernoi terapii* (Biological Models and Physical Mechanisms of Laser Therapy), Vladivostok: Dal'nauka, 2002.
5. Gibbons, I.R., *J. Cell Biol.*, 1984, vol. 91, no. 1, p. 107.
6. Shkuratov, D.Yu., Chudnovskii, V.M., and Drozdov, A.L., *Tsitologiya,* 1997, vol. 39, no. 1, pp. 25–28.
7. Karu, T.I., *Photochem. Photobiol.*, 2008, vol. 84, no. 1, pp. 1091–1099.
8. Drozdov, A.L. and Vinnikova, V.V., *Ontogenez*, 2010, vol. 41, no. 1, pp. 47–57.
9. Cosson, J., Croison, A.-L., Suqnet, M., Fauvel, C., Dreanno, C., and Billard, R., *Reproduction*, 2008, vol. 136, pp. 277–294.

Translated by M. Batrukova