ISSN 1990-7478, Biochemistry (Moscow), Supplement Series A: Membrane and Cell Biology, 2018, Vol. 12, No. 1, pp. 43–49. © Pleiades Publishing, Ltd., 2018.
Original Russian Text © A.A. Dzhatdoeva, E.V. Proskurnina, A.M. Nesterova, I.V. Dubinkin, T.V. Gaponova, S.I. Obydenny, Yu.A. Vladimirov, 2017, published in Biologicheskie Membrany, 2017, Vol. 34, No. 6, pp. 116–123.

ARTICLES

Mitochondria as a Source of Superoxide Anion Radical in Human Platelets

A. A. Dzhatdoeva[a, *], E. V. Proskurnina[a], A. M. Nesterova[b], I. V. Dubinkin[c], T. V. Gaponova[c], S. I. Obydenny[d], and Yu. A. Vladimirov[a, b]

[a] *Moscow Lomonosov State University, Faculty of Fundamental Medicine, Moscow, 117192 Russia*
[b] *Federal Research Centre 'Crystallography and Photonics', Russian Academy of Sciences, Moscow, 119333 Russia*
[c] *National Research Center for Hematology, Ministry of Health of Russian Federation, Moscow, 125167 Russia*
[d] *Dmitry Rogachev National Center of Pediatric Hematology, Oncology, and Immunology, (NC-PHOI), Moscow, 117198 Russia*
**e-mail: ayshatdj@gmail.com*

Received February 2, 2017; in final form, June 23, 2017

Abstract—The radical-producing activity of human platelets has been studied using the enhanced chemiluminescence method. It is shown that chemiluminescence of isolated platelets is observed only in the presence of lucigenin, a selective probe for superoxide anion; the luminescence is amplified many times upon the addition of NADH and NADPH, the substrates of oxidative chains. The chemiluminescence is not affected by diphenyliodonium, an inhibitor of NADPH oxidase, but it is inhibited in a dose-dependent manner by the oxidative phosphorylation uncouplers dinitrophenol and rotenone. Thus, a superoxide anion radical is the main free radical generated by platelets, and mitochondria are one of the superoxide anion radical sources in platelets.

Keywords: platelets, enhanced chemiluminescence, reactive oxygen species
DOI: 10.1134/S1990747818010051

INTRODUCTION

There are several major systems in the cells, in which free radicals (reactive oxygen species, ROS) are formed as a main or by-product. NADPH oxidases, membrane enzymes producing superoxide anion radical (SAR) in the reaction of one-electron reduction, are on the first place. Seven isoforms of this enzyme complex are known, of which phagocytic Nox2 is studied best of all. Many cells, including endothelial cells and fibroblasts, use NADPH oxidase for ROS signaling pathway and cross-interactions (crosstalk) with the immune system cells [1].

Mitochondria are an important SAR-producing system. It is known that there are 11 sites in the inner membrane, where SAR leakage takes place as the result of the NADH-dependent chain work, mainly in Complexes I, II, and III; SAR is produced both in the matrix and in the intermembrane space [2]. In addition to the mitochondrial respiratory chain, a significant role in the SAR production belongs to the microsomal respiratory chain (cytochrome P-450 system) generating the amount of ROS comparable to the amount of ROS generated in mitochondria [3]; the microsomal system in the liver cells is especially important.

The SAR-producing enzyme xanthine oxidase is expressed in the form of xanthine dehydrogenase and then is converted into xanthine oxidase as a result of posttranslational modification. This enzyme is involved in the metabolism of purines, oxidizing various substrates, primarily xanthine and hypoxanthine, to form SAR [4].

It has recently been proved by EPR that peroxyl radicals are intermediate products in the arachidonic acid (AA) conversion catalyzed by cyclooxygenase [5]. AA can also be metabolized by endoplasmic reticulum monooxygenases.

All these free radical sources are present in platelets, particularly cyclooxygenase, and theoretically can make a contribution to ROS production. As early as in 1977, Marcus showed that platelets are capable of producing ROS, however, there are still different opinions about their origin [6, 7]. Both non-activated platelets and platelets activated with such agonists as collagen and thrombin produce ROS, including SAR, hydroxyl radical, and hydrogen peroxide [8–11]. The endogenous synthesis of ROS points to their autocrine or paracrine role in the platelet activation, similar to that described for exogenous ROS [12].

Platelets contain NADPH oxidase; many of its subunits were detected in platelets [13, 14]. Platelet

Nox2 NADPH-oxidase generates little extracellular ROS as compared to neutrophils; the produced SAR amount is of the order of nmol/min/10^5 cells, which is approximately the same as in the endothelial cells, but is only 1% of the SAR released from activated neutrophils [15]. This makes it important for intracellular signaling mechanisms, rather than for the body's protective response, although the respiratory burst is enhanced by the presence of platelets [13, 16–18]. Platelet NADPH oxidase is stimulated by collagen and thrombin.

The inhibition of platelet SAR formation by diphenyliodonium chloride has been shown independently by several research groups [13, 14]. Krötz et al. [13] showed that chimeric peptide gp91 ds-tat, which specifically inhibits the interaction between subunits gp91 and p47, also prevents the SAR production by platelets.

There is evidence that xanthine oxidase in platelets contributes to the thrombin-induced ROS production [13]. According to other observations, an additional role belongs to the phospholipase A_2-dependent AA release [19, 20]. Other enzymes of the AA metabolism, such as lipoxygenase, can participate in the ROS release, too [21, 22], but up to now there are no data on the functional role of lipoxygenase-and cyclooxygenase-dependent ROS formation in platelets.

In sum, the NADPH oxidase system is presently regarded the main ROS source in platelets, although xanthine oxidase also may contribute. Little attention was paid to other ROS sources: mitochondria, microsomal respiratory chain, and the AA cascade. The aim of this work was to investigate possible ROS sources in platelets.

MATERIALS AND METHODS

Chemiluminescence (CL) was recorded with a Lum-1200 12-channel chemiluminometer (DISoft, Russia). Isolated platelets were placed into a chemiluminometer cuvette and their own CL was recorded for 5 min. Then the chemiluminescence enhancer was added and the signal was recorded for 10 min, then either NADH or NADPH (Sigma–Aldrich, USA) dissolved in bidistilled water (Milli-Q) was added. The total system volume was 1.0 mL. All experiments were performed in Hanks solution (Paneko, Russia, pH 7.4) with the addition of HEPES (Sigma, USA).

The following stock solutions were prepared: 1 mM luminol (Sigma–Aldrich, USA) in phosphate buffered saline (PBS); 0.5 mM rhodamine 6G (Reakhim, Russia) in bidistilled water (Milli-Q); 1 mM lucigenin (Sigma–Aldrich, USA); 0.5 mM coumarin C-525 (NIOPIK, Russia), and coumarin C-334 (Sigma, USA) in methanol (Merck, Germany).

The following reagents were used: superoxide dismutase (SOD from bovine erythrocytes, 5030 U/mg of protein, Sigma, USA); an uncoupler of oxidative phosphorylation, 2, 4-dinitrophenol (DNP, Sigma, USA); an inhibitor of Complex I, rotenone (Sigma, USA), and the NADPH oxidase inhibitor diphenyliodonium chloride (Fluka, USA).

Methanol (Merck, Germany) and dimethyl sulfoxide (DMSO, Helicon, Russia) were used as solvents.

Microscopy. The permeation of the CL probes into platelets was studied by confocal microscopy on a Zeiss Hal device 100, the wavelength of the excitation laser was 405 nm, the fluorescence emission was recorded at 520 ± 17 nm.

Platelets were isolated from the whole blood (the blood of a practically healthy donor was obtained from NC-PHOI) stabilized with citrate in the presence of prostaglandin E1 (final concentration, 1 μM) and apyrase (final concentration, 0.1 U/mL). The first centrifugation was carried out at 100 *g* for 8 min, the platelet-rich plasma was separated from the erythrocyte-leukocyte sediment, threefold volume of 3.8% citrate was added, centrifuged for 5 min at 400 *g*, and the upper layer of plasma was separated from the sediment of platelets, which was resuspended in a buffer to the opalescent solution state [23].

A 30-μL aliquot of the platelet suspension was applied to the fibrinogen-modified glass and mixed with an equal volume of the CL probes. After 10 min, the solution was carefully removed from the substrate; adhered platelets were washed several times with HEPES-supplemented Hanks solution and analyzed using the microscope.

Isolation of platelets from the platelet-rich plasma. Platelet-rich plasma of healthy donors for the CL experiments was provided by the Hematology Research Center, Ministry of Health of the Russian Federation. Overall 25 samples of platelet-rich plasma of practically healthy donors were investigated. Three parallel measurements were performed for each sample. During the experiments, the plasma was stored at room temperature and constant swinging for no more than 3 h. The platelet-rich plasma was centrifuged at 1000 *g* for 20 min at 25°C. The pellet was resuspended in the Hanks/HEPES solution, pH 7.4, in a volume equal to the volume of the original plasma and centrifuged at 1000 *g* for 15 min at 25°C. The platelet washing procedure was repeated 2 times. The platelet count in the investigated samples was controlled using an Abacus Junior 30 hematological analyzer (Diatron, Austria). The suspension of isolated platelets contained about 6–8 × 10^{11} platelets/L.

Isolation of mitochondria from rat liver. Mitochondria were isolated from the liver of male Wistar rats (275 ± 25 g) according to the procedure described in [24]. The rats were decapitated under ether anesthesia. All procedures were carried out at a temperature of 0–4°C on ice. A liver sample of about 4 g was washed in an isolation buffer containing 10 mM HEPES, 1 mM EGTA, 70 mM sucrose, and 200 mM mannitol, pH 7.5 (all reagents from Sigma–Aldrich, USA). The

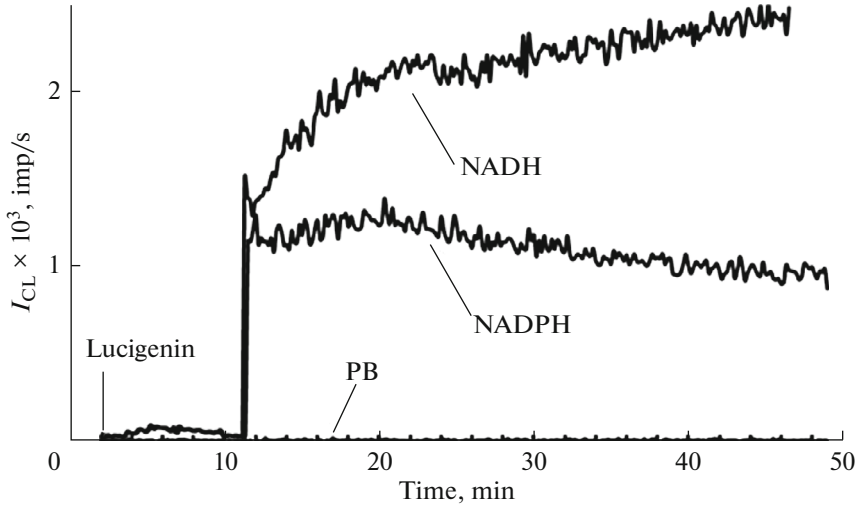

Fig. 1. Chemiluminescence (CL) of isolated platelets stimulated by NADH and NADPH in the presence of 200 μM lucigenin. The time of lucigenin addition and the development of own CL is indicated by the arrow. Phosphate buffer solution (PBS) was added as a control. The volume of the system was 1.0 mL: 800 μL of a platelet suspension in a Hanks/HEPES solution and 0.2 mL of the enhancer, 0.1 mM NADH. Cell concentration was approx of 4×10^{11} platelets/L.

sample was homogenized in 40 mL of the same buffer containing 1 mg/mL of delipidized bovine serum albumin (Sigma–Aldrich, USA). The resulting suspension was centrifuged at 600 *g* for 5 min. The supernatant was separated and centrifuged at 11000 *g* for 10 min. The supernatant was discarded and the sediment was resuspended in 40 mL of the isolation buffer. The resulting suspension was centrifuged at 600 *g* for 5 min. The supernatant was separated and centrifuged at 11000 *g* for 10 min. The supernatant was removed, and the sediment was resuspended in the storage buffer containing 10 mM HEPES, 250 mM sucrose, 1 mM ATP, 0.08 mM ADP, 5 mM sodium succinate, and 2 mM KH_2PO_4, pH 7.5, at 0–4°C on ice.

The mitochondrial protein concentration was determined by the standard method with Total protein reagent (Sigma, USA) using bovine serum albumin as a standard. In all experiments mitochondria were used with the protein concentration of approximately 10 mg/mL.

The mitochondrial viability was monitored by the electrochemical measurement of the mitochondrial respiratory control (Expert, Ekoniks, Russia). The respiratory control in all experiments ranged from 4 to 6.

The samples were centrifuged in a Hermle Z 32 HK centrifuge (Hermle Labortechnik, Germany) with cooling.

RESULTS AND DISCUSSION

The effect of CL activators and influence of NADH and NADPH. Various chemical and physical activators of CL were used to elucidate the nature of free radicals produced by platelets. Luminol was used as a nonselective activator for estimating the total free radical production; lucigenin was used as a probe for SAR. The contribution of the lipid radical formation was evaluated with rhodamine 6G and quinolizin coumarins C-334 and C-525 (Fig. 1). Weak own CL of isolated platelets was observed only in the presence of lucigenin.

Significant increase of CL was observed upon the addition of reduced NADH or NADPH (0.1 mM) in the presence of lucigenin (Fig. 1); the luminescence remained at the background level in the presence of other CL probes. Phosphate buffer was used as a control solution.

The degree of CL increase (the ratio of the stimulated CL stationary intensity to the level of spontaneous luminescence) depends on a particular blood sample and the state of the platelets, but NADH was several times more effective stimulus than NADPH in all studied cases ($n = 25$).

The effect of inhibitors on induced ROS production. The effect of SOD on the NADH-stimulated chemiluminescence development in the isolated platelets was investigated in a series of experiments. Different amounts of SOD were added to the system containing approximately 3.2×10^8 isolated platelets, 0.2 mM lucigenin, and 0.1 mM NADH (Fig. 2).

The addition of SOD led to a sharp drop of the chemiluminescence intensity; and the form of the CL curve did not depend on the concentration of the added enzyme (Fig. 2a). The CL suppression reached a plateau (Fig. 2b), indicating that some SAR produced in the mitochondria flowed out from the platelets.

The effect of the respiratory systems inhibitors on SAR production. The addition of NADPH oxidase inhibitor diphenyliodonium (DPI) of to the platelets/lucigenin/NADH and platelets/lucigenin/NADPH CL systems even at a concentration of 1 mM exerted

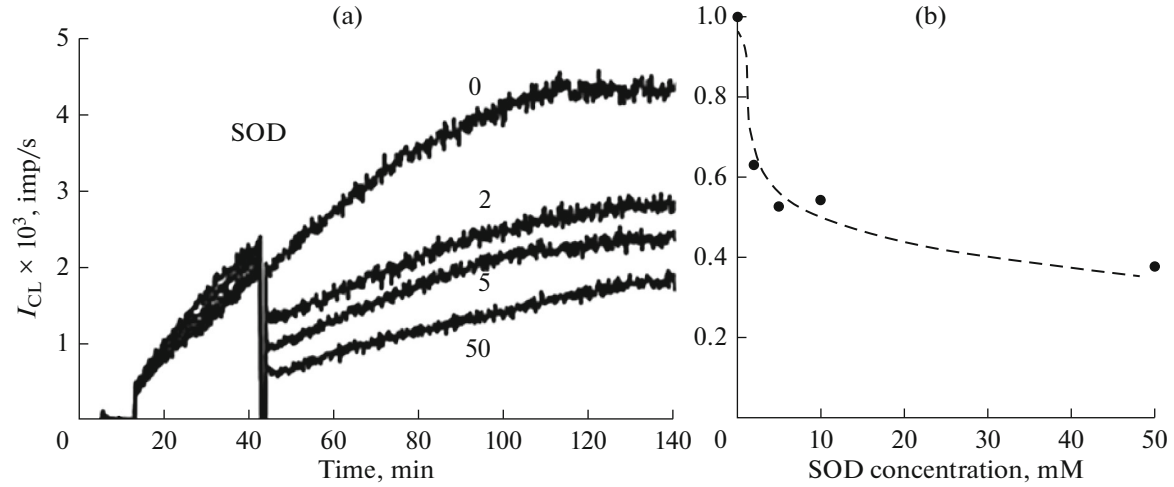

Fig. 2. The lucigenin-activated chemiluminescence (CL) quenching of isolated platelets stimulated by various concentrations of NADH and superoxide dismutase (SOD). (a) CL curves; the arrow indicates the time of SOD addition, the numbers at the curves indicate a SOD concentration, μM; (b) The degree of CL suppression calculated as a ratio of the total CL intensity for 2 h after SOD addition to the control value.

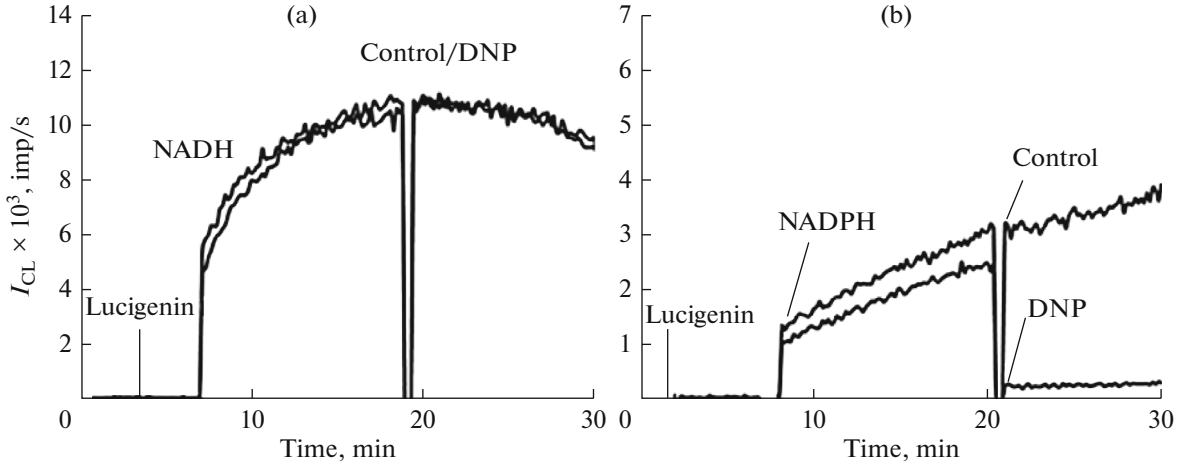

Fig. 3. Additions to the system: (a) 0.1 mM diphenyliodonium (DPI) to the isolated platelets stimulated by NADH (0.1 mM) does not change lucigenin-CL as compared to the control experiment; *arrow* indicates the time of addition of Hanks solution (control) and diphenyliodonium; (b) 2 mM 2,4-dinitrophenol (DNP) inhibits lucigenin-enhanced CL stimulated by NADH. Concentration of lucigenin was 2 mM and that of NADH, 0.1 mM.

no effect on CL (Fig. 3a). A respiratory chain Complex I inhibitor rotenone does not affect NADH- and NADPH-stimulated CL (data are not shown, the curves were similar to those in Fig. 3), in contrast to DNP, an uncoupler of oxidative phosphorylation (Fig. 3b).

NADH-stimulated lucigenin CL as a method of studying the SAR production by mitochondria. Isolated mitochondria were investigated to interpret the data on the lucigenin-activated platelet chemiluminescence. Mitochondria are known to actively generate SAR mainly by the respiratory chain located in the inner membrane [25], predominantly by NADH ubiquinone oxidoreductase (respiratory Complex I). NADPH-dependent oxidative chain located in the outer mitochondrial membrane [26] is capable of producing free radicals [27].

As in the series with platelets, NADH, NADPH, or phosphate buffer as a control were added to isolated mitochondria (Fig. 4).

The NADH addition to the mitochondria led, as in the case of isolated platelets, to a sharp increase of the lucigenin-enhanced CL intensity, but it was followed by a slow decline. This difference of the CL kinetics for mitochondria and platelets may be explained by a higher concentration of mitochondria in the system, which rapidly utilized oxygen and substrate. After NADPH addition, CL tended to a stationary level as in the case of platelets. It follows from Fig. 4 that NADH

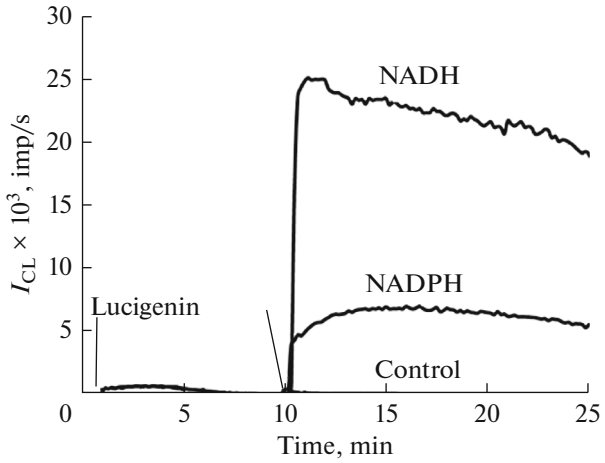

Fig. 4. The CL response of isolated mitochondria to the action of NADH and NADPH. Phosphate buffer solution was added as a control. The system includes: 800 μL of mitochondria (10 mg/mL), 0.2 mM lucigenin, and 0.1 mM NADH or NADPH. *Arrow* indicates the time of the NADH or NADPH application.

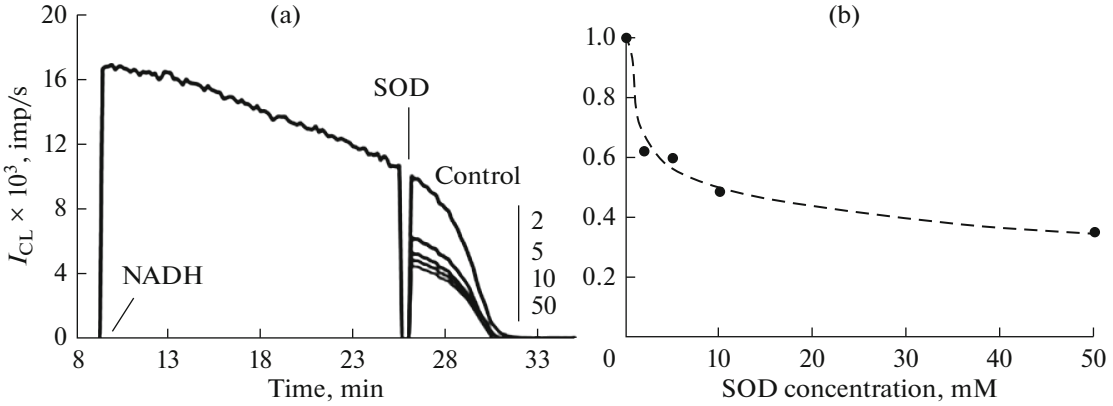

Fig. 5. The lucigenin-CL quenching of isolated mitochondria stimulated by NADH and superoxide dismutase at various concentrations. (a) The CL curves; the arrow indicates the time of SOD addition, the numbers at the curves indicate the SOD concentration, μM; (b) Decrease in the light sum of chemiluminescence measured for 2 h after the addition of SOD as compared to the control without SOD.

stimulates SAR production in mitochondria at an order of magnitude stronger than NADPH.

NADH fluorescence measurement (λ_{ex} = 340 nm, λ_{em} = 460 nm) showed that the CL decay is due to the NADH depletion. The signal acquires a stationary character with the decrease of the number of mitochondria in the system; the repeated NADH addition after its partial expenditure restores CL to the initial level.

CL of both mitochondria and platelets is quenched by SOD in a dose-dependent manner (Fig. 5). The concentration curve is characterized by saturation, which implies that part of the SAR may not be neutralized by SOD, i.e., SAR is intra-mitochondrial.

Diphenyliodonium at a concentrations below 0.1 mM does not affect lucigenin-activated chemiluminescence of mitochondria, while DNP at a concentration of 2 mM almost completely inhibits SAR production. According to both these indicators, the SAR production in rat liver mitochondria and in human blood platelets did not differ.

Lucigenin entrance into platelets. The data on the ability of lucigenin to enter into cells are controversial, and there are no data about its permeation into platelets. It is shown by the confocal microscopy that, after incubation of isolated platelets with lucigenin on a fibrinogen-coated glass (10 min), lucigenin enters into the platelets, inhomogeneously filling the entire volume (Fig. 6).

Similar experiments with the addition of NADH and NADPH showed that these stimuli exert no effect on the lucigenin permeation into the platelets. The permeation of the stimuli into the platelets requires an additional study, but on the basis of some preliminary experiments it can be concluded that these substances

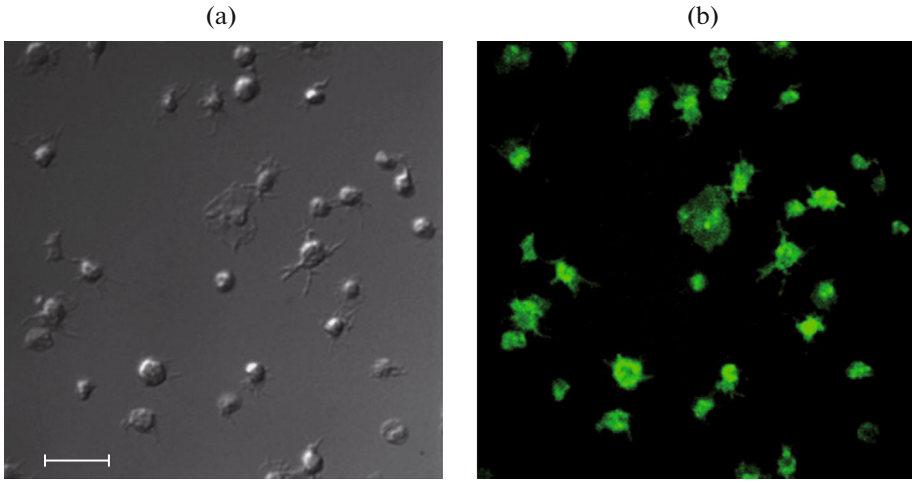

Fig. 6. A confocal microscopy image of the platelets incubated with lucigenin. (a) Differential interference contrast; (b) Fluorescence excited at 405 nm, recorded at 520 ± 17 nm. Scale bar, 10 μm.

enter into the platelets through the plasma membrane in small amounts.

CONCLUSIONS

At present, there is no doubt that the blood cells located in the parietal blood flow, platelets and neutrophils, exchange information with each other and with endothelial cells in the language of chemical mediators, including ROS, and the platelets are not only a target of free radicals but are also their source. It is believed that platelets generate SAR by the membrane NADPH oxidase Nox2 complex, possibly due to the cytoplasmic xanthine oxidase.

However, platelets contain a countable number of mitochondria (from 3–4 to 10), which, nevertheless, occupy a significant portion of the volume of such a small cell as a platelet (approx. 3–4 μm in size) and which, therefore, can serve as an important source of SAR. Lucigenin is considered to be a selective probe for SAR [28], so it is actively used to study ROS production by NADPH-oxidase and mitochondria [29].

Neither luminol nor coumarin nor rhodamine responded to the NADH- and NADPH-stimulated platelets; therefore, it can be concluded that lipid peroxidation with the formation of excited ketone, from which energy is transferred to coumarins, does not occur upon this stimulation; hydrogen peroxide is not formed, either, as is evidenced by the absence of the luminescence with luminol. It can be assumed that SAR is formed, because a weak lucigenin-enhanced luminescence is observed, which is multiply enhanced upon the addition of NADH and NADPH; their mitochondrial source may also be assumed, since positively charged lucigenin is accumulated in mitochondria. A similar NADH and NADPH stimulation effect was obtained using lucigenin-activated chemiluminescence of isolated mitochondria.

Using confocal microscopy, we showed that during incubation under the conditions of our experiment lucigenin entered into the platelet and was distributed relatively homogeneously throughout its volume. So, indeed, lucigenin CL can be generated within the platelets. The question of whether NADH and NADPH permeate into the platelets remains open, but it is known that NADH can enter, for example, into astrocytes using a receptor [30].

Incomplete CL quenching by SOD suggests that a part of SAR exits from the platelets and another part remains inside the mitochondria of these cells. The lack of the inhibitory effect of diphenyliodonium puts in question the assertion that NADPH oxidase is the primary SAR source in platelets. DNP, an oxidative phosphorylation uncoupler, inhibits SAR formation in the same manner both in platelets and mitochondria. Therefore, it may be assumed that mitochondria are at least one of the SAR sources in platelets.

The presented approach makes it possible not only to assess the ROS-producing function of mitochondria in the platelets and to study their influence on the processes of activation and aggregation, but also to use platelets as a kind of “laboratory animals” available from the blood in the assessment of radical-producing function of mitochondria in, for example, neurons of the central nervous system. Attempts have recently been made to use this approach, particularly in psychiatry for studying the pathogenesis of endogenous psychoses [31]. In the future, it seems expedient to study the radical-producing effect of platelet activators such as collagen, ADP, and thrombin; and also to examine the platelets of a representative group of healthy donors to determine the reference interval of the ROS-producing function normal values of platelets.

REFERENCES

1. Panday A., Sahoo M.K., Osorio D., Batra S. 2015. NADPH oxidases: An overview from structure to innate immunity-associated pathologies. *Cell Mol. Immunol.* **12**, 5–23.
2. Kozjak-Pavlovic V. 2017. The MICOS complex of human mitochondria. *Cell Tissue Res.* **367** (1), 83–93.
3. Bae Y.S., Oh H., Rhee S.G., Yoo Y.D. 2011. Regulation of reactive oxygen species generation in cell signaling. *Mol. Cells.* **32**, 491–509.
4. Labat-Robert J., Robert L. 2014. Longevity and aging. Role of free radicals and xanthine oxidase. A review. *Pathol. Biol. (Paris).* **62**, 61–66.
5. Gu Y., Xu Y., Law B., Qian S.Y. 2013. The first characterization of free radicals formed from cellular COX-catalyzed peroxidation. *Free Radic. Biol. Med.* **57**, 49–60.
6. Pratico D., Pasin M., Barry O.P., Ghiselli A., Sabatino G., Iuliano L., FitzGerald G.A., Violi F. 1999. Iron-dependent human platelet activation and hydroxyl radical formation: Involvement of protein kinase C. *Circulation.* **99**, 3118–3124.
7. Caccese D., Pratico D., Ghiselli A., Natoli S., Pignatelli P., Sanguigni V., Iuliano L., Violi F. 2000. Superoxide anion and hydroxyl radical release by collagen-induced platelet aggregation—role of arachidonic acid metabolism. *Thromb. Haemost.* **83**, 485–490.
8. Ferroni P., Vazzana N., Riondino S., Cuccurullo C., Guadagni F., Davi G. 2012. Superoxide anion and hydroxyl radical release by collagen-induced platelet aggregation—role of arachidonic acid metabolism. *Thromb. Haemos.t* **83**, 485–490.
9. Finazzi-Agro A., Menichelli A., Persiani M., Biancini G., Del Principe D. 1982. Hydrogen peroxide release from human blood platelets. *Biochim. Biophys. Acta.* **718**, 21–25.
10. Loscalzo J. 1985. N-Acetylcysteine potentiates inhibition of platelet aggregation by nitroglycerin. *J. Clin. Invest.* **76**, 703–708.
11. Wachowicz B., Olas B., Zbikowska H.M., Buczynski A. 2002. Generation of reactive oxygen species in blood platelets. *Platelets.* **13**, 175–182.
12. Krotz F., Sohn H.Y., Pohl U. 2004. Reactive oxygen species: Players in the platelet game. *Arterioscler. Thromb. Vasc. Bio.l* **24**, 1988–1996.
13. Krotz F., Sohn H.Y., Gloe T., Zahler S., Riexinger T., Schiele T.M., Becker B.F., Theisen K., Klauss V., Pohl U. 2002. NAD(P)H oxidase-dependent platelet superoxide anion release increases platelet recruitment. *Blood.* **100**, 917–924.
14. McVeigh G.E., Hamilton P., Wilson M., Hanratty C.G., Leahey W.J., Devine A.B., Morgan D.G., Dixon L.J., McGrath L.T. 2002. Platelet nitric oxide and superoxide release during the development of nitrate tolerance: Effect of supplemental ascorbate. *Circulation.* **106**, 208–213.
15. Lassegue B., Clempus R.E. 2003. Vascular NAD(P)H oxidases: Specific features, expression, and regulation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **285**, R277–297.
16. Begonja A.J., Gambaryan S., Geiger J., Aktas B., Pozgajova M., Nieswandt B., Walter U. 2005. Platelet NAD(P)H-oxidase-generated ROS production regulates alphaIIbbeta3-integrin activation independent of the NO/cGMP pathway. *Blood.* **106**, 2757–2760.
17. Chlopicki S., Olszanecki R., Janiszewski M., Laurindo F.R., Panz T., Miedzobrodzki J. 2004. Functional role of NADPH oxidase in activation of platelets. *Antioxid. Redox Signal.* **6**, 691–698.
18. Zalavary S., Grenegard M., Stendahl O., Bengtsson T. 1996. Platelets enhance Fc(gamma) receptor-mediated phagocytosis and respiratory burst in neutrophils: The role of purinergic modulation and actin polymerization. *J. Leukoc. Biol.* **60**, 58–68.
19. FitzGerald G.A. 2003. COX-2 and beyond: Approaches to prostaglandin inhibition in human disease. *Nat. Rev. Drug Discov.* **2**, 879–890.
20. Niwa K., Haensel C., Ross M.E., Iadecola C. 2001. Cyclooxygenase-1 participates in selected vasodilator responses of the cerebral circulation. *Circ. Res.* **88**, 600–608.
21. Jahn B., Hansch G.M. 1990. Oxygen radical generation in human platelets: Dependence on 12-lipoxygenase activity and on the glutathione cycle. *Int. Arch. Allergy Appl. Immunol.* **93**, 7-3-79.
22. Singh D., Greenwald J.E., Bianchine J., Metz E.N., Sagone A.L., Jr. 1981. Evidence for the generation of hydroxyl radical during arachidonic acid metabolism by human platelets. *Am. J. Hematol.* **11**, 233–240.
23. Gilbert G.E., Sims P.J., Wiedmer T., Furie B., Furie B.C., Shattil S.J. 1991. Platelet-derived microparticles express high affinity receptors for factor VIII. *J. Biol. Chem.* **266**, 17261–17268.
24. Storrie B., Madden E.A. 1990. Isolation of subcellular organelles. *Meth. Enzymol.* **182**, 203–225.
25. Jezek P., Hlavata L. 2005. Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism. *Int. J. Biochem. Cell. Biol.* **37**, 2478–2503.
26. Sottocasa G.L., Kuylenstierna B., Ernster L., Bergstrand A. 1967. An electron-transport system associated with the outer membrane of liver mitochondria. *J. Cell. Biol.* **32**, 415–438.
27. Archakov A.I. 1975. *Mikrosomal'noe okislenie* (Microsomal oxidation). M.: Nauka.
28. Aasen T.B., Bolann B., Glette J., Ulvik R.J., Schreiner A. 1987. Lucigenin-dependent chemiluminescence in mononuclear phagocytes. Role of superoxide anion. *Scand. J. Clin. Lab. Invest.* **47**, 673–679.
29. Kruglov A.G., Yurkov I.S., Teplova V.V., Evtodienko Y.V. 2002. Lucigenin-derived chemiluminescence in intact isolated mitochondria. *Biochemistry (Moscow).* **67**, 1262–1270.
30. Lu H., Burns D., Garnier P., Wei G., Zhu K., Ying W. 2007. P2X7 receptors mediate NADH transport across the plasma membranes of astrocytes. *Biochem. Biophys. Res. Commun.* **362**, 946–950.
31. Dietrich-Muszalska A., Kwiatkowska A. 2014. Generation of superoxide anion radicals and platelet glutathione peroxidase activity in patients with schizophrenia. *Neuropsychiatric Dis. Treat.* **10**, 703–709.

Translated by E. Puchkov