

## Literature

PHYSIOMED ELEKTROMEDIZIN AG

ADDRESS  
Hutweide 10  
91220 Schnaittach/Laipersdorf  
Germany

PHONE +49 (0) 91 26 / 25 87-0  
FAX +49 (0) 91 26 / 25 87-25  
E-MAIL [info@physiomed.de](mailto:info@physiomed.de)  
WEB [www.physiomed.de](http://www.physiomed.de)

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Institution:

Dept. Molecular Biology & Dpt. Physiology, Russian State University, Moscow, Russia

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### I. EFFECTS OF DEEP OSCILLATION® ON THE WHOLE BLOOD AND WHITE BLOOD CELL CHEMILUMINESCENCE

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The stimulation of oxygen radical production by normal white blood cells either isolated or in the presence of other blood components (whole blood) is assumed as additional activation of the immune defense against bacterial and viral infections. The process of free radical release from the blood leukocytes is tightly interconnected with the increase of Ca<sup>2+</sup> transport into cells. The weak electro-magnetic field seems to activate Ca-transport in various types of cells. Whole blood and white blood cells generated oxygen species were studied by luminol-dependent chemiluminescence. 10 µl of freshly drawn human blood were mixed with 0.980 ml of Hanks' balanced salt solution containing 5 x 10<sup>-5</sup> M luminol. To isolate total pool of white blood cells (WBC), 1 ml of freshly drawn blood was laid on the Hipaque gradient,  $\rho = 1,119$ . After centrifugation at 250 x g for 60 min at room temperature, the pellet containing WBC was washed twice in a big volume of cold Hanks' balanced salt solution. Finally, washed WBC were re-suspended in 0.1 ml of HBSS supplied with fetal calf serum. Always 10<sup>6</sup> WBC were added to a chemiluminometer cuvette (LKB 1251, Wallach Oy, Finland). The free radical generation was induced by adding 10 µl of PMA (phorbol myristate acetate, final concentration, 10 nM). The chemiluminescence response to the phorbol ester was monitored for 30 min at  $t = 37^\circ\text{C}$  and continuous stirring. The whole blood or isolated WBC were placed into plastic tube and exposed for DEEP OSCILLATION® field for a certain period of time, after which the chemiluminescence measurements were performed. The regime of exposure was as following: in cycles, one cycle with increasing frequency from 10 to 100 Hz during 1 sec. Then, the steady 100 Hz frequency for 60 sec. Finally, a slow decrease (30 sec) in the frequency to the background one of 10 Hz

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### II. EFFECTS ON THE ADP-INDUCED PLATELET DEGRANULATION

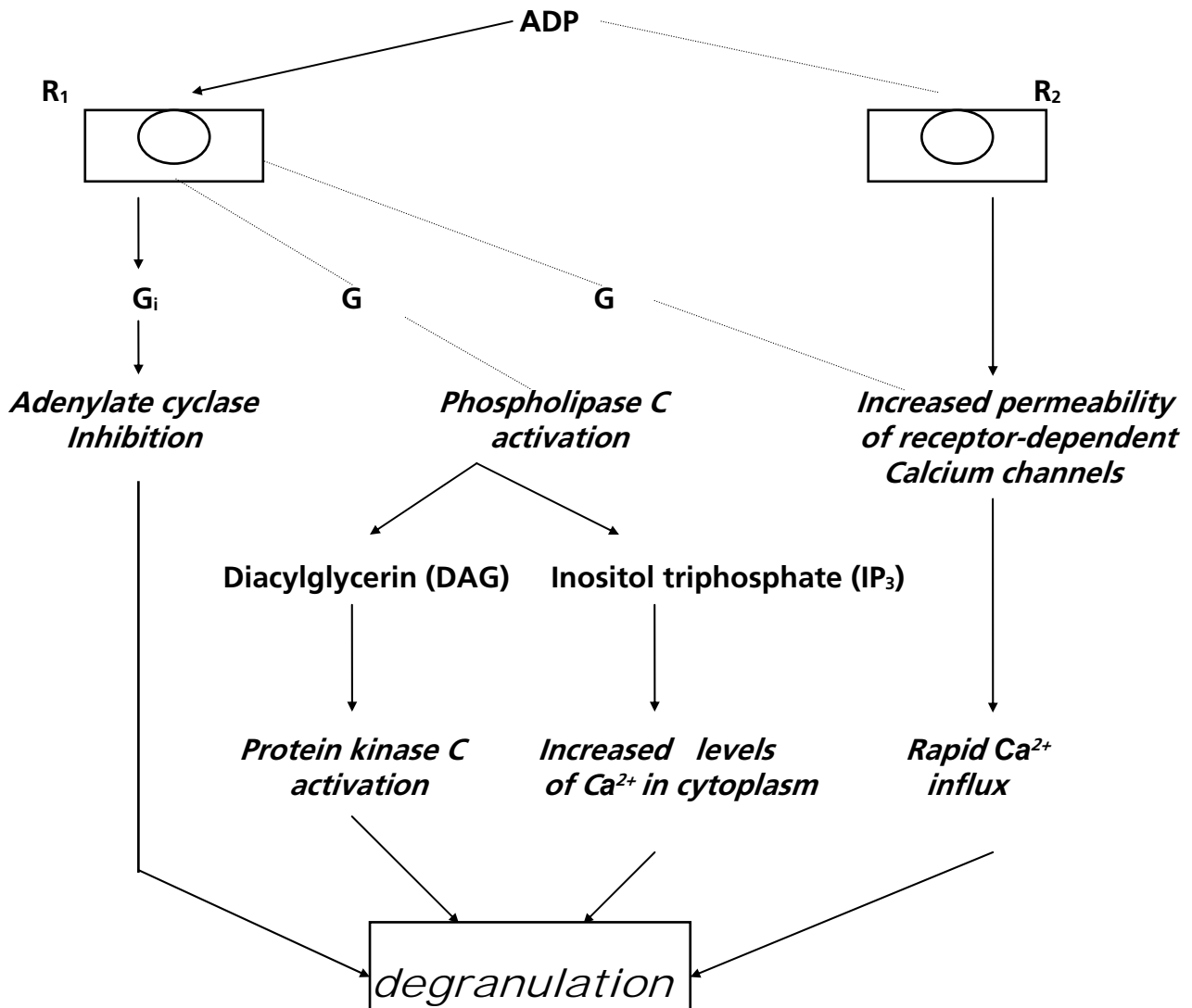
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It is thought that all agonists of platelet aggregation/degranulation, known so far, increase the intracellular concentration of calcium ions. Calcium ions are essential for the activation of phospholipase A<sub>2</sub> and protein kinase C (through diacyl-glycerin), and the following phosphorylation of intracellular proteins. Upon activation of the platelet enzyme phospholipase A<sub>2</sub>, free arachidonic acid is formed and then, being oxidized by cyclooxygenase it gives rise of prostaglandin H<sub>2</sub> and tromboxan A<sub>2</sub>. These two latter active compounds seem to play a key role in the secondary platelet aggregation and secretion. The increase of intracellular Ca<sup>2+</sup> leads also to the activation of calmodulin-regulated kinase of the light

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myosine chain. As a result, the phosphorylation of myosine molecule occurs followed by rounding of the platelet, the pseudopodia forming, the platelet granule centralization and release (degranulation). The mechanism of ADP-induced platelet activation and aggregation is shown at the scheme 1 [1].

Scheme 1. The hypothetical mechanisms of ADP-induced platelet degranulation.



R1 – ADP receptor regulated by G-protein;  
 R2 – ADP receptor, which regulates calcium channels;  
 Gi – inhibiting G-protein;  
 DAG- diacyl-glycerin;  
 PI3 – inositol-1,4,5-triphosphate.

Solid and dashed lines show the known and hypothetical activation pathways, respectively.

In general, platelet degranulation allows suggesting that the acting factor(s) induce the platelet functions, which is of great importance for the wound healing process. It is well

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known that the process starts from the platelet activation, platelet-derived cytokine formation, degranulation, and aggregation to stop bleeding and facilitate the tissue remodeling process. These are suggested physiological functions of the platelets. On the other hand, the process of platelet intra-vascular activation is a clear symptom of clot formation. Therefore, excessive platelet activation is not desirable in the individuals at risk of thrombosis. Since a weak electro-magnetic field is thought to open cellular Ca-channels, it's logical to suggest that DEEP OSCILLATION® treatment could result in the moderate platelet activation.

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## Materials and Methods

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The platelet-rich rabbit plasma was used to evaluate the effects of DEEP OSCILLATION® on the platelet degranulation. Briefly, the platelet-rich plasma was obtained from freshly drawn rabbit venous blood by mixing it with 3.8% sodium citrate solution (9:1 w/w, respectively). The mixture was centrifuged at 460 x g for 20 min. The supernatant was collected and immediately used for the degranulation measurements. The platelet degranulation was registered on a Chronolog "Platelet ionized calcium lumi-aggregometer" (Chrono-log Co., USA) using luciferine-luciferase system as a tool for analyzing of ATP granule release from the activated platelets [2]. All the measurements were performed at 37°C and continuous mixing. One ml of platelet suspension (before or after exposure to DEEP OSCILLATION® field for a certain period of time. The regime of exposure was the same as in the experiments with the whole blood) was added to a cuvette of lumi-aggregometer and incubated for five minutes to balance cells at 37°C. Then, 10 µl of ADP solution (final concentration 10 µM) was added and the light emission was continuously recorded. The results are expressed as mV/10<sup>9</sup> platelets.

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## Results and Discussion

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### a) Oxygen radical production by white blood cells

The results obtained showed that DEEP OSCILLATION® did not affect the free radical release from isolated WBC (the data not shown). In contrast, DEEP OSCILLATION® exposure did increase free radical production by the whole blood leukocytes stimulated by the phorbol ester PMA (Table and Fig.1-3). The maximal stimulation (about 40-45%) was observed after exposure for 3 min, 15 min, and 30 min. When stimulated with the calcium channel opener (Ca-ionophore A23187), the whole blood leukocytes produced a short spike of luminol-dependent chemiluminescence, which was slightly inhibited by the exposure to DEEP OSCILLATION® for periods of time from 3 to 30 min (Table and Fig. 4-6, see EXCEL file "Combined results").

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## Conclusions

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On the basis of the results obtained in the in vitro experiments we concluded:

1. The exposure of isolated white blood cells to the electrical field produced by DEEP OSCILLATION® did not change the biochemical pathways, which lead to oxygen radical production. Therefore the weak electrical signals with particular characteristics did not influence WBC cellular membranes directly.
2. The exposure of whole blood activated by the protein kinase C activator PMA to the DEEP OSCILLATION® produced electrical field for 3, 15, and 30 min led to significant increase (40-45%) of oxygen radical production. By unknown yet mechanisms, DEEP OSCILLATION® involves the blood plasma components in the WBC membrane enzyme activation, which is a key enzyme in oxygen radical generation by the blood leukocytes. Since PMA simulates one of the most important endogenous defense mechanisms against bacterial, viral, and cancer cells (endogenous immunity process) we concluded that DEEP OSCILLATION® acts as an immunostimulator enhancing the body capacity to fight exogenous infections and, probably, to diminish the risk of tumor development.
3. Exposure to DEEP OSCILLATION® slightly inhibited oxygen radical production by the whole blood leukocytes activated by Ca-ionophore A 23187. The activation with A23187 results in the release of inflammatory mediators such as the products of arachidonic acid oxidation. Therefore DEEP OSCILLATION® could be considered as having an anti-inflammatory action.
4. The incubation of platelets under exposure to a weak electrical field for 3-30 min did not affect either platelet degranulation (Fig. 7) or aggregation (data not shown). Therefore DEEP OSCILLATION® did not have any effect on the major platelet functions in the in vitro experiments. On this basis we could suggest that DEEP OSCILLATION® exposure would not increase the risk of thrombosis in the individuals.