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Volume-dependent regulation of the respiratory burst of activated human neutrophils

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Abstract. The effect of incubation medium osmolality on the respiratory burst of human neutrophils was studied using luminol-dependent chemiluminescence (CL) as an indicator of burst activity. Neutrophils were stimulated with N-formyl-Met-Leu-Phe (FMLP), phorbol-12-myristate-13-acetate (PMA), the calcium ionophore A23187, thermoaggregated IgG (IgG_n), and opsonized zymosan (OZ). It was shown that increasing the osmolality of the incubation medium from 320 up to 420 mosM decreased the A23187- and OZ-induced CL responses by 90%. Under the same conditions PMA-, FMLP- and IgG_n-induced CL responses were decreased by 40–60%. A decrease of osmolality to 200 mosM resulted in a 2–3 fold decrease of the A23187-, PMA- and FMLP-induced CL and in a 60–80% increase of OZ- and IgG_n-induced CL. It is suggested that osmolality-mediated alteration of cell volume is an important mechanism for regulating neutrophil activity.

Key words. Neutrophil leukocytes; luminol-dependent chemiluminescence; cell volume.

A wide range of stimulators as well as contact with foreign agents lead to neutrophil chemotaxis, degranulation, phagocytosis and a burst of oxidative metabolism (respiratory burst). The latter is a sharp increase in molecular oxygen consumption and production of highly reactive metabolites: superoxide-anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^\cdot$) and singlet oxygen ($^1O_2$) through activation of an NADPH-oxidase. Active oxygen species play a major role in the bactericidal action of neutrophils and contribute to the role of these cells in inflammatory processes[1].

The influence of hypo- and hypertonic medium conditions on the respiratory burst of neutrophils seems to be significant, particularly for understanding the relationship between changes in cellular volume and functional activity in various pathological states. In asthma, for example, alterations of osmolality can lead to induction of bronchoconstriction[2]. In cystic fibrosis the drastic alteration of osmolality in airway ducts may be due to the decreased activity of Cl^- channels in secretory epithelium cells[3].

It was shown that neutrophil shrinkage induced by hyperosmotic medium is accompanied by regulatory volume increase due to activation of Na^+/H^+ exchange[4]. Swelling-induced regulatory volume decrease was also observed in these cells and this phenomenon was accompanied by activation of Ca^{2+}-induced K^+ channels and the conductive Cl^- channels[5].

Only a few data concerning osmotic regulation of the neutrophil respiratory burst can be found in the literature, largely representing research on the influence of hyperosmotic solutions used in peritoneal dialysis[6,7] and the influence of hypoosmotic conditions on production of O_2^- by peritoneal guinea pig neutrophils[8].

Our paper presents a thorough study of the influence of medium osmolality on the ability of human neutrophils to produce reactive oxygen metabolites in response to a wide range of activating agents: formyl peptide f-Met-Leu-Phe (FMLP), phorbol-12-myristate-13-acetate (PMA), calcium ionophore A23187, opsonized zymosan (OZ), and thermoaggregated IgG (IgG_n).

Materials and methods

Chemicals. Dextran T 500, Ficoll-Paque (Pharmacia, Sweden); FMLP, PMA, A23187, human IgG (Serva, FRG); zymosan (Sigma, USA); other chemicals were all of analytical grade.

Neutrophils. Heparinized (50 units/ml) venous blood was obtained from adult donors. Neutrophils were isolated by the standard procedure of dextran sedimentation followed by Ficoll-Paque gradient centrifugation[9]. Residual erythrocytes were removed by isotonic lysis using 0.83% NH_4Cl[10]. The cells were washed and finally suspended (2×10^7 cells/ml) in isoosmotic (320 mosM) medium A consisting of (mM): 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 1 Na_2HPO_4, 5 glucose, 10 HEPES-tris, pH 7.4, and were kept on ice. About 97% of the cell suspension was neutrophils. Vital dye trypan blue added to the suspension was excluded by 99% of the cells, a generally accepted criterion of integrity.

Measurement of CL. Production of active oxygen metabolites during neutrophil activation was measured by the method of luminol-dependent chemiluminescence using the chemiluminometer PCL (Moscow, Russia) at

37 °C. The reaction mixture contained medium of different osmolality (pH 7.4) with a final volume of 1 ml, 10^{-4} M luminol and 2×10^5 neutrophils. To activate the system FMLP (10^{-5} M), PMA (10^{-8} M), A23187 (10^{-5} M), IgG_n (300 µg/ml) or OZ (100 µg/ml) were added and light emission was recorded. In the case of treatment of neutrophils with FMLP, the cell suspension was kept for 30 min at 37 °C in advance.

Incubation medium. Hypotonic medium (200–300 mosM) was prepared by decreasing the Na^+ concentration in medium A. Hypertonic medium (345–520 mosM) was prepared by adding sucrose to medium A. The value of osmolality was counted as for ideal solutions.

Zymosan opsonisation. Zymosan (10 mg/ml) was opsonized by incubation with 50% (v/v) fresh human serum with medium A at 37°C for 30 min. The particles were then washed 3 times and resuspended in medium A to achieve a final concentration of 10 mg/ml.

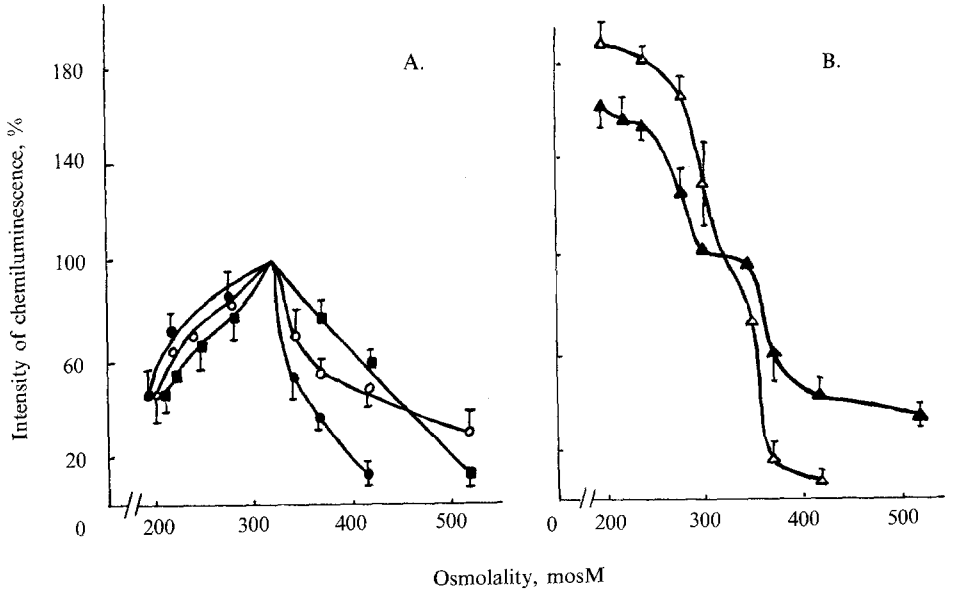
Aggregation of IgG. For aggregation by heating, human IgG was incubated at 10 mg/ml in 0.15 M NaCl in a 63°C water bath for 30 min and chilled on ice[11].

Results and discussion

Changes in medium osmolality did not influence the kinetics of the CL response, but modulated its intensity. The dose-dependence of the amplitudes are of special interest, because even a slight shift in osmolality (25–40 mosM) seems to be sufficient for significant alteration in activated CL intensity (see fig.).

Exposure of cells to hypertonic conditions results in a decrease in CL signal regardless of the activator used. More impressive decreases were observed in the case of A23187 and OZ activation. For these substances, an increase of osmolality from 320 to 420 mosM results in a decrease in CL intensity of up to 90%. Such dependence conforms with data in the literature. Using a hypertonic solution for peritoneal dialysis, Duwe et al.[6] showed that increasing the osmolality from 275 to 475 mosM resulted in almost complete suppression of CL of peripheral human blood leucocytes stimulated with opsonized *E. coli*. In an analogous experiment Zhou et al.[7] demonstrated dose-dependent inhibition of O_2^- production by neutrophils from canine and human blood stimulated with opsonized zymosan: increasing the osmolality from 284 to 442 mosM led to a two-fold decrease in O_2^- production.

However, a clear interpretation of these results is difficult because glucose was used to increase the osmolality of the medium. Unlike sucrose, glucose can penetrate the plasma membrane which can result in modification of the energetic metabolism of the cell. Increased intracellular glucose concentrations also lead to an increase in cytoplasmic osmolality, which can partially smooth the action of hypertonic solutions on neutrophil volume. Moreover O_2^- production was measured after 15 min of incubation of cells in hyperosmotic medium. As shown by Grinstein et al.[4] this is enough time for recovery of about 15% of original cellular volume due to activation of Na^+/H^+ exchange. Our experiments allow for direct estimation of the influ-



The effect of osmolality of incubation medium on the intensity of chemiluminescence of human neutrophils activated by FMLP: 10^{-5} M (■), PMA: 10^{-8} M (○), A-23187: 10^{-5} M (●), agg IgG: 300 µg/ml (▲) and OZ: 100 µg/ml (△). Intensity of chemiluminescence is given as the percentage of that gene rated by cells in isotonic (320 mosM) medium. The results are given as the means ±S.D. for four experiments.

ence of changes in osmolality on cellular volume and functional activity of neutrophils because 1) using sucrose to increase osmolality excludes intracellular side-effects, and 2) activated CL was measured 1–2 min after exposure of cells to hyperosmotic conditions, when regulatory compensation of cell volume has hardly begun. Our data also show that decreasing medium osmolality to 200 mosM leads to a 2-3-fold decrease in CL response to stimulation with FMLP, PMA and A23187, but to an increase of up to 60–80% upon stimulation with OZ and IgG_n. By contrast, Hiura et al.[8] found an increase in O_2^- production upon stimulation of guinea pig peritoneal neutrophils with the PMA analogue 1-oleoyl-2-acetylglycerol. This discrepancy can most probably be explained by species-specificity of neutrophils. For example, the dependence of O_2^- production on medium Na^+ content differs sharply for guinea pig and human neutrophils[12].

The opposite influence of hypoosmolality on CL induced by FMLP, PMA and A23187 on the one hand and OZ and IgG_n on the other is probably the consequence of different modes of respiratory burst activation. Mechanisms for the modulative influence of cell volume on neutrophil activation have not been investigated. It may be that it is accomplished by changes in cellular metabolism, for there are indications of an increase in phosphatidic acid[8] or a decrease in FMLP-induced free calcium[5] during exposure of neutrophils to hypotonic conditions.

The available data indicate that the respiratory burst in neutrophils is highly sensitive to changes in intracellular volume. By contrast, varying the osmolality from 200 to 700 mosM failed to reveal any essential influence on the phosphoinositide response of cultured smooth muscle cells activated by angiotensin-2 (ref. 13). Significant modification of ion-transport activity occurs in the same cell line with osmolalities above 400 and below 200 mosM[13]. Activation of Na^+, K^+-cotransport in rate erythrocytes was observed for osmolality increases to 420 mosM, with the maximal response at 620 mosM. Under the same conditions, Na^+/H^+ exchange was activated above 520 mosM, with a maximal effect at 720 mosM[14].

The present paper demonstrates that medium osmolality plays an essential role in regulating the respiratory burst of human neutrophils. The form of this dependence varies significantly among activators. From a pathophysiological point of view, this is especially important if we take into consideration that in lung tissue these cells (and also macrophages) are very close to epithelial cells, in which transcellular currents of Na^+, K^+ and Cl^- determine the osmolality of the alveolar fluid. Possibly such an interaction between epithelial cells and cells (neutrophils) producing an inflammatory reaction can explain the bronchodilatory effect of furosemide[15], which inhibits the main route of transcellular currents of Na^+, K^+ and Cl^--Na^+, K^+, $2Cl^-$-cotransport.

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