Effect of the Osmotic Pressure of the Medium on the Chemiluminescence of Human Neutrophils

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A broad spectrum of stimulators or contact with foreign agents induce the chemotaxis, degranulation, phagocytosis, and remodeling of oxidative metabolism in neutrophils (Np), resulting in an "oxidative burst" that includes a dramatic rise in the consumption of molecular oxygen and activation of NADPH-oxidase, followed by the formation of active oxygen metabolites: superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$), and singlet oxygen (O_2^{*}). Active oxygen forms play the main role in the bactericidal action of Np, thereby defining the role of the latter in inflammatory reactions [12].

In this connection the effect of the hypo- and hypertonic environment on the Np oxidative burst is of special interest. In the field of basic research this question is important for the elucidation of the relationship between the change of volume and functional activity of the cell. The clinical aspect concerns the study of the role of Np in various pathological processes, e.g., bronchial asthma, where a change of osmolarity can induce bronchospasm [7].

There are very few reports regarding the osmotic regulation of the Np oxidative burst. They include studies of the effect on human Np activation of hyperosmotic solutions used in peritoneal dialysis [2,16], and of the effect of hypoosmotic conditions on $O_2^{\cdot-}$ generation in guinea pig peritoneal Np [5].

This report presents a detailed study of the influence of different osmotic conditions on the ability of human Np to produce highly reactive oxygen metabolites in response to a broad spectrum of activating agents: formyl-peptide (f-Met-Leu-Phe [FMLP], phorbol-12-myristate-13-acetate (PMA), A-23187 calcium ionophore, opsonized zymosan, and thermoaggregated IgG (aggIgG).

MATERIALS AND METHODS

Heparinized (heparin 50 U per ml) fresh blood of healthy donors was used. The separation of Np was performed after Boyum [1], including procedures of dextran sedimentation and Ficoll-Paque gradient (1.077 g per ml) centrifugation. The remaining erythrocytes were eliminated by isoosmotic lysis with 0.83% NH_4Cl [15]. The cells were suspended in medium A (140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM Na_2HPO_4, 5 mm glucose, 10 mM HEPES-Tris; pH 7.4), adjusted to a concentration of 2×10^7 cells per ml and stored on ice. The prevalence of Np in the resulting suspension was up to 97%; Np viability as determined by staining with Trypan Blue was 99%.

Generation of active forms of oxygen was registered in luminol-dependent chemiluminescence (CL) assay using a PKhL-01 chemiluminometer. The reaction was conducted at 37°C in 1 ml of medium (pH 7.4) containing 2×10^5 and 10^{-4} luminol.

A hypoosmotic solution (200-300 mosM) was obtained by diluting medium A with analogous medium without NaCl. A hyperosmotic solution (345-720 mosM) was prepared by adding sucrose to medium A. The osmolarity indexes were calculated as for ideal solutions.

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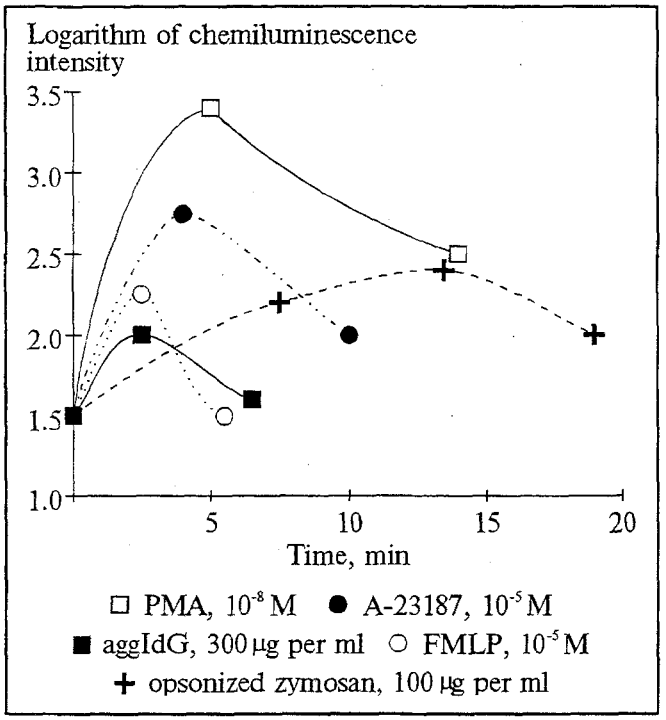

Fig. 1. Kinetics of human Np chemiluminescence.

Cells were activated with the following agents: 10^{-5} M FMLP (Serva), 10^{-8} M PMA (Serva), 10^{-5} M A-23187 (Serva), human serum-opsonized zymosan (Sigma) 100 μg per ml, and thermoaggregated IgG 300 μg per ml. In experiments with FMLP activation Np were preincubated for 30 min at 37°C before the addition of activator.

Zymosan (10 mg per ml) was opsonized by incubation for 30 min at 37°C in normal human serum diluted one-half with medium A. After three washings, the opsonized zymosan was suspended in the same medium up to a concentration of 10 mg per ml. Human IgG (Serva) was dissolved in 0.15 M NaCl (10 mg per ml) and aggregated for 30 min at 63°C with subsequent rapid cooling on ice [4].

RESULTS

Figure 1 illustrates typical kinetic curves of CL induced by activated human Np. Analysis of the results shows that both the response kinetics and intensities differ from each other and depend on the activator used, which is in agreement with published data [6,14]. The osmotic changes did not affect the CL-response kinetics, but did modulate its intensity, as shown in Fig. 2. The striking dependence of amplitude on the osmotic level is of special interest, as a minimal shift toward increase or decrease of the osmotic pressure (by 25-40 mosM) was sufficient to change considerably the intensity of activated CL.

Exposure of cells to hypertonic conditions leads to weakening of the CL signal irrespective of the nature of the activator. The most marked sensitivity to the increase of osmotic tonicity is observed in experiments with A-23187 and opsonized zymosan. When these agents are used as activators, the increase of osmolarity from 320 to 420 mosM leads to a 90% reduction of oxidative burst intensity. Such a dependence is consistent with published reports. For instance, in experiments with a hypertonic solution for peritoneal dialysis, Duwe *et al.* [2] showed that an osmolarity increase from 275 to 475 mosM almost totally inhibited the CL of human peripheral blood leukocytes stimulated with opsonized *E. coli*. In similar experiments Zhou *et al.* [16] demonstrated a dose-dependent inhibition of $O_2^{\cdot-}$ generation in human and canine blood Np stimulated with opsonized zymosan. For instance, an osmolarity increase from 284 to 442 mosM resulted in a twofold reduction of $O_2^{\cdot-}$ formation. However, we think that an unequivocal interpretation of the reported data is complicated, as glucose was used to increase the tonicity of the solution. Glucose, unlike sucrose, can pass through the plasma membrane and modify the energetic metabolism in the cell. The increase in intracellular glucose leads to an increase of cytoplasmic osmolarity and therefore partly mitigates the effect of the hypertonic solution on the Np volume. Moreover,

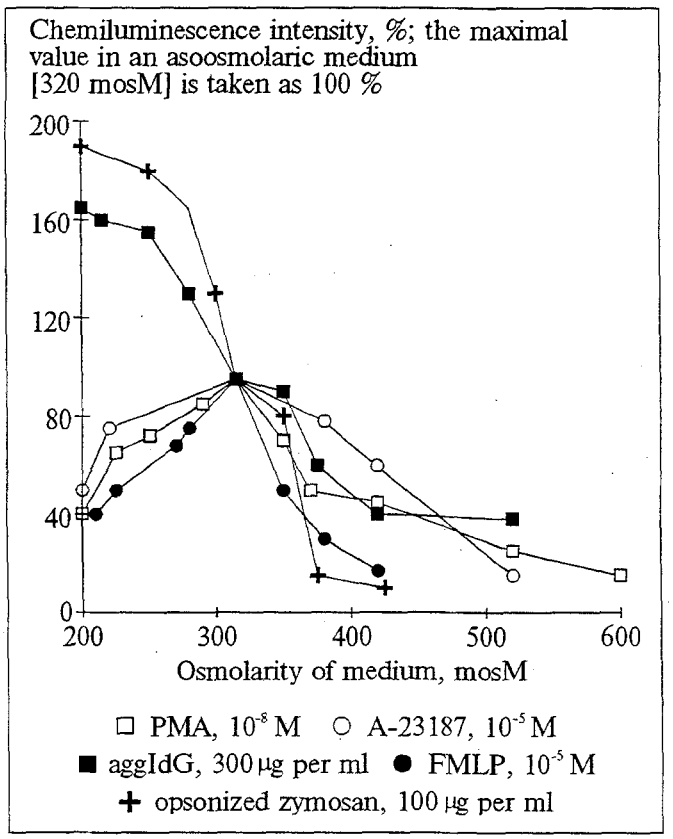

Fig. 2. Effect of osmolarity of medium on intensity of human Np chemiluminescence. The arithmetic mean values of 4 experiments are presented.

the authors registered $O_2^{\cdot-}$ formation 15 min after the completion of Np treatment with hypertonic solution. As shown by Grinstein *et al.* [3], human Np are capable of reconstituting up to 15% of the initial volume during such a time interval. In this respect our experiments enable us to directly assess the effect of the osmotic change and of the consequent cell volume change on the functional activity of Np, since, in the first place, the use of sucrose to increase the osmolarity makes it possible to avoid side effects upon the cell and, second, we registered activated CL as early as 1-2 min after cell exposure to the hyperosmotic conditions, when the regulatory reconstitution of the volume was virtually absent.

Our results also show that hypoosmotic treatment of Np leads to a two- to threefold inhibition of the CL response to FMLP, PMA, and A-23187; however, the response to opsonized zymosan and aggIgG rose by 60-80%. When comparing these results with the literature, it is interesting to note that in the report of Hiura *et al.*, on the contrary, an increase of $O_2^{\cdot-}$ formation was observed in guinea pig peritoneal Np in response to 1-oleil-2-acetylglycerol, a PMA analog, under hypotonic conditions. Such a difference may be due to species-specific features of Np. For instance, the dependence of $O_2^{\cdot-}$ formation on the Na^+ content in the medium is different for guinea pig [5] and human [13] Np.

Opposite effects of hypoosmotic conditions on CL induced, on the one hand, by FMLP, PMA, and A-23187 and, on the other, by opsonized zymosan and aggIgG may be due to the existence of different pathways of oxidative burst activation. The mechanisms of the effect of cell volume on Np activation are unclear. One can assume that they are mediated via a change in cell metabolism, as there are indications of a rise of the phosphatidic acid level [5] or a diminishment of the FMLP-induced calcium signal [8] in Np under hypotonic conditions.

According to available data, the oxidative burst in Np is most sensitive to a change of the volume of the reactions. For example, in our experiments varying the osmolarity in the range of 200 to 700 mosM failed to produce a considerable effect on the angiotensin-2-induced phosphoinositide response in the cultured smooth-muscle cells [10]. However, an osmolarity of more than 400 mosM and/or less than 250 mosM substantially modified the activity of the ion-transfer system in these cells [10]. A reliable activation of Na^+, K^+-cotransfer in rat erythrocytes was observed for an osmolarity increased to 420 mosM, the maximum response being attained at 620 mosM. Under the same conditions the Na^+/H^+ exchange was activated beginning from an osmolarity of 520 mosM, and the maximum response was registered at an osmolarity of 720 mosM [9].

Thus, it has been shown in this report that the osmolarity of a medium exerts a considerable effect upon the oxidative burst generation in Np. The pattern of this relationship substantially varies depending on the nature of the activator. This is especially important from the pathophysiological point of view if we remember that in the lung tissue Np and related cells, namely macrophages, reside in close proximity to the epithelial cells, wherein the Na^+, K^+, and Cl^- transcellular flows determine the osmolarity of the alveolar fluid. Perhaps, precisely this aspect of epithelial and inflammation-mediating cell interaction explains the bronchodilatory effect of furosemide [11], as the latter is known to inhibit the main pathway of the Na^+, K^+, and Cl^- transcellular flow via Na^+, K^+, and $2Cl^-$-cotransfer.

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