INTRODUCTION

H$_2$O$_2$ released from activated leukocytes kills bacteria and tumor cells. However, adjacent cells (endothelial cells and blood cells) also may serve as a targets for H$_2$O$_2$ which may kill these cells or change their functions [1]. In the present work we have studied some functional charges induced by H$_2$O$_2$ in human endothelial cells, platelets and neutrophils.

MATERIALS AND METHODS

Human endothelial cells (EC) were obtained from umbilical vein by proteolytic treatment and cultivated in Medium 199 with 20% serum. Platelets (platelet-rich-plasma, PRP) were obtained by centrifugation from fresh human blood. Neutrophils were separated from the same source by centrifugation in Ficoll-Paque and sedimentation in Dextran.

Assay of cytotoxic action of H$_2$O$_2$ on EC and PMN was performed using specific release of $^{51}$Cr. Concentration of inositolphosphates (IP$_1$ and IP$_3$) in EC pre-loaded with myo-[2-$^3$H]-inositol was determined by ion-exchange chromatography of cell lysates on Dowex I. Platelet aggregation was studied in a standard aggregometer. Generation of superoxide anion by PMN was assessed by reduction of Cytochrome C.

RESULTS AND DISCUSSION

Activation of PI-turnover in EC by sublethal doses of H$_2$O$_2$.

H$_2$O$_2$ at doses 1,000 mM and higher induces rapid massive EC death. Doses lower 100 mM does not kill EC. A dose of 100 mM does not induces $^{51}$Cr release during 3-4 h after treatment, while 20 h later death of 50% cells was revealed. Therefore doses lower than 100 mM are sublethal; 100 mM is pre-lethal for some hours.

Incubation with 50-150 mM of H$_2$O$_2$ results in 2-5 fold increase in concentration of inositolphosphates IP$_1$ and IP$_3$ in EC. The effect was rapid (10 min) and reversible (two hours later IP$_1$ and IP$_3$ decreased to control level). Inositolphosphates are the secondary messengers regulating Ca$^{2+}$ flux in cell [2]. Agonists bind with receptors and via protein-G mediated pathway activate membrane-associated phospholipase C which cleaves phosphoinositides in the cell membrane [2]. However, in contrast with activation of PI-turnover by histamine, H$_2$O$_2$ induced activation was not affected by specific
regulators of G-proteins - stable GTP analogues. This fact support receptor-independent action of hydrogen peroxide on PL-C.

Histamine-included activation was two-fold greater than H$_2$O$_2$-induced. Pre-incubation with H$_2$O$_2$ lead to fast reduction of consequent histamine-induced response (to 50% reduction 10 min after treatment with H$_2$O$_2$). Therefore, hydrogen peroxide at sublethal doses may activate PI-turnover and change hormonal status of EC, probably via desensitization-like pathway.

Activation of cytotoxicity and PI-turnover by H$_2$O$_2$ were dependent on the Ca$^{2+}$ input into the EC. Ca$^{2+}$-blocker verapamil and Ca$^{2+}$-chelator EGTA protect EC from killing by H$_2$O$_2$: 10 ± 5% and 7 ± 3% of dead cells, resp., vs 65 ± 10% of dead cells in control 3 h after treatment with 300 mM of H$_2$O$_2$. EGTA and verapamil eliminate H$_2$O$_2$-induced activation of PI-turnover in EC completely. Therefore, PI-activation by sublethal doses of H$_2$O$_2$ is G-independent protein, but Ca$^{2+}$-dependent.

Modulation of the platelet function by hydrogen peroxide.

Aggregometry of human PRP shows that PRP preincubation with 1 mM of H$_2$O$_2$ leads to reduction of the platelet aggregation induced by ADP and collagen. In ADP-induced aggregation H$_2$O$_2$ almost completely inhibits the second phase of aggregation. In collagen-induced aggregation both stages were inhibited drastically.

In contrast to inhibition by pretreatment, simultaneous addition of the same dose of H$_2$O$_2$ leads to enhancement of ADP induced aggregation. Moreover, H$_2$O$_2$ addition at the plateau phase of aggregation induces the "second wave" of aggregation. Thus, H$_2$O$_2$ has a bimodal effect on platelet function. Accounting abovementioned data about PI-turnover activation in EC and consequent reduction of histamine -induced response, we may propose that H$_2$O$_2$ induces the similar reaction in platelets and thus enhances response to receptor-mediated stimulus at simultaneous treatment, but decrease it at pre-treatment. Desensitization-like mechanisms may be involved in this effect. Direct action of H$_2$O$_2$ on metabolism of arachidonic acid and its derivatives also may be involved in the mechanism.

Priming of human neutrophils to FMLP by hydrogen peroxide.

PMN involved in preformed inflammatory focus also may became target for exogenous H$_2$O$_2$. Assay of 51Cr release shows that human PMN are extremely resistant to cytotoxic action of H$_2$O$_2$: high doses as 30 and 100 mM induce fast death of 50-70% of cells. To reveal death induced by 1 and 10 mM additional incubation for 3-4 h is necessary. Thus, H$_2$O$_2$-producing cells are more resistant than "innocent bystanders."

We studied the influence of H$_2$O$_2$ on the O$_2$ generation by PMN in response to PMA and FMLP. Both agents act via the activation of the protein kinase C. FMLP action is receptor and G-protein-mediated, while PMA acts directly. At toxic doses 30-100 mM H$_2$O$_2$ inhibits O$_2$ generation in response to both agents and percent of inhibition was equal to percent of dead cells. Dose 10 mM H$_2$O$_2$ at non-lethal time 30 min after treatment enhance O$_2$ generation twice in response to FMLP, but not to PMA. Thus H$_2$O$_2$ primes PMN specifically to receptor mediated respiratory burst.

Therefore, H$_2$O$_2$ at sublethal doses changes cellular responses to agonists in various cells playing