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MICROBIOLOGY AND IMMUNOLOGY

Phosphoinositide Metabolism in Endothelial Cells of Human Umbilical Vein Effected by *Y. pestis* Toxin

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Histamine stimulates the metabolism of inositol phosphates and raises the level of prostacycline in endothelial cells. The activatory effect of histamine markedly decreases when incubation is performed in the presence of PT, whereas the baseline level of phosphoinositide metabolites does not change in endothelial cells.

Key Words: *plague toxin; endothelium of human umbilical vein; phosphoinositide metabolism*

Thrombohemorrhagic syndrome, or the syndrome of disseminated intravascular coagulation (DIC) is characteristic for plague and plague intoxication [1,3]. The damage inflicted on endothelial cells (EC) both by the pathogenic organism itself and by its toxins is one of the numerous factors which provoke the development of DIC in bacterial intoxications [3]. The biochemical mechanisms underlying the damaging effect of *Y.pestis* toxins are not clear. It is known that the regulation of vascular tonus and of thromboresistance is mediated by neurotransmitters and hormones via specific receptors which are situated on the outer surface of endothelial cell membranes [13]. In addition to cAMP-regulating receptors there are so-called Ca-mobilizing receptors [4,12], whose effect is mediated by the hydrolysis of phosphatidylinositol-4,5-biphosphate (IP) to yield inositol-1,4,5-triphosphate (IP3) and 1,2-diacylglycerol (DAG). IP3 increases the intracellular concentration of Ca due to its mobilization from the intracellular depot [4,6,7]. DAG is the activator of proteinkinase C and is a source of arachidonic acid, which metabolizes into prostacycline, thromboxane, and other eicosanoids in EC [9].

In this study we examined the effect of plague toxin (PT) on phosphoinositide metabolism (PIM)

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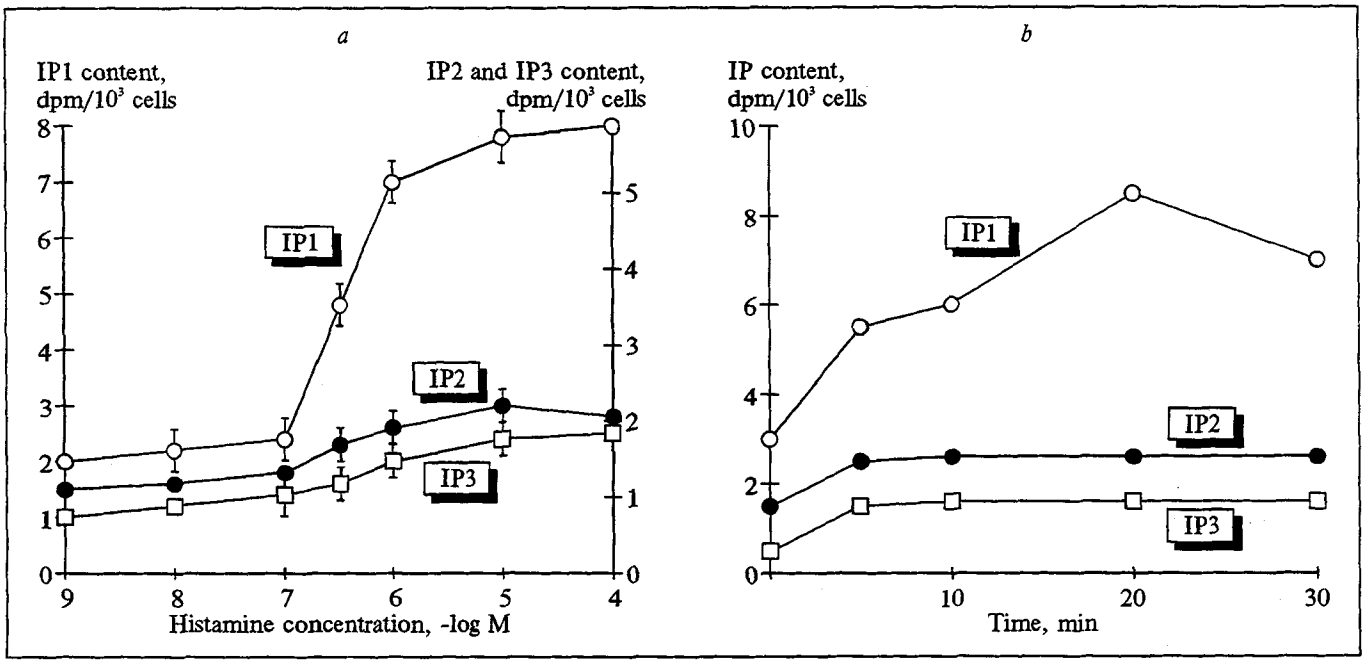

Fig. 1. Effect of histamine on concentration (*a*) and time course (*b*) of accumulation of PIM products in human endothelial cells.

and on the content of prostacycline in endothelial cells of human umbilical vein.

MATERIALS AND METHODS

Toxin of EV-76 plague microbe strain № 1290 (fraction II) was manufactured at the Rostov-on-Don Antiplague Research Institute.

A culture of endothelial cells from human umbilical vein was isolated and characterized as described elsewhere [5]. The monolayer cell culture was obtained after 7-8 passages and did not contain fibroblasts or smooth muscle cells.

For measurement of PIM products the monolayer cell culture (10^6 cells/well) was incubated for 48 h at 37° C with myo-[2-^{3}H]-inositol in the absence of endothelial growth factor in medium 199 [11]. PT was added to cells in medium containing 10mM HEPES-NaOH (pH 7.5), 140 mM NaCl, 2.8 mM KCl, 2 mM $MgCl_2$, and 1 mM $CaCl_2$, and incubation was performed for 60 min at 37°C; 0.1 mM histamine was added to the cells 5 min prior to the termination of incubation. The reaction was stopped by the addition of 1 ml boiling 1% SDS (w/v) /30 mM EDTA solution per well. The well contents were stirred, heated for 5 min at 96° C, and then 3 ml H_2O were added. The well contents were applied to columns containing 0.5 ml of Dowex 2×4cm; 200-400 mesh formate form. The columns were rinsed with 14 ml water for the removal of myo-[^{3}H]-inositol. Inositol phosphate esters were eluted by a stepwise gradient (7 ml) of ammonium formate [7,11]. Radioactivity was assayed using a liquid scintillat-

TABLE 1. Effect of PT on the Level (in dpm/10^3 cells) of Inositol Phosphates in Endothelial Cells of Human Umbilical Vein ($M \pm m$)

Additives	IP1	IP2	IP3
Control	3210±430	1732±191	984±120
Histamine (10^{-4} M)	5646±850*	2578±318*	1763±210*
PT (60 μg/ml)	3690±59	1651±8	928±101
PT (120 mg/ml) + histamine	3390±269**	1602±143**	966±24**
PT (60 μg/ml) + histamine	4238±489**	2059±230**	1125±130**
PT (6 μg/ml) + histamine	4835±520*	2490±312*	1611±116*
PT, t° (120 μg/ml) + histamine	3215±430	1569±173	1043±143

Note. Asterisks indicate significance ($p \leq 0.05$) between inositol phosphate contents: * – in control and test cells, ** – in cells in the presence of histamine and in cells treated with histamine and PT.

ing counter and expressed as dpm (the number of radioactive decays per min) per 10^3 cells. Prostacycline was measured by radioimmune assay according to the amount of stable analog 6-keto-$PGF1_{\alpha}$, using enzyme kits (Budapest).

RESULTS

Histamine activated the metabolism of IP in EC dose-dependently via the H_1 histamine receptors (Fig.1, *a*). The maximal activation of PIM was observed in human EC at 10^{-4} M histamine. It was noted that the concentration of IP2 and IP3 increased during the first minute and leveled toward the 5th min. The accumulation of IP1 required somewhat more time (Fig. 1, *b*).

A concentration of PT as high as 60 μg/ml does not change the level of inositol phosphates in cells incubated with toxin for 60 min (Table 1), nor does it after the morphology of the endothelium. Histamine (10^{-4} M) in a course of incubation with cells for 5 min activated IP1, IP2, and IP3 synthesis by 76, 49, and 79%, respectively. However, preincubation of endothelial cells for 60 min with the addition of PT induced a decrease of the activatory effect of histamine on the level of inositol phosphates. It is to be noted that the inhibitory effect depended on the dose of PT (Table 1), so that at a toxin concentration as high as 120 μg/ml the activating effect of histamine was fully blocked.

Toxin inactivated by heating (100° C, 15 min) produced the same decrease of the activating effect of histamine on the level of inositol phosphates as the initial preparation of toxin (Table 1).

Thus, a decrease of the level of PIM products owing to the *Y.pestis* toxin may result in a lowering of hormone-induced Ca^{2+} release from the endoplasmic reticulum and of the entry of this cation from the outside into the cell [4,6,7]. The decrease in the concentration of intracellular free Ca^{2+} may in turn be a "trigger" for changing the conditions of functioning of numerous Ca-dependent enzymes, such as phospholipase A_2, which catalyzes the release of arachidonic acid from cell phospholipids [10]. On the other hand, a decrease in the rate of IP hydrolysis may cause a drop in the content of DAG, which is also a source of the synthesis of thromboxane, prostaglandins, and other eicosanoids [5-7]. It is known that many substances able to activate PIM in EC increase the content of different classes of prostaglandins in endothelium [8,9].

In our experiments histamine [10^{-4} M] increased 5.1-fold the concentration of prostacycline in the control cells (15.2±2.1, control; 77.5±10.2 ng/ml in the presence of histamine, $p<0.05$). After incubation of the cell culture in the presence of 60 mg/ml PT for 60 min, histamine increased the formation of prostacycline only 2.4-fold (36.4±5.8 ng/ml, $p<0.05$) as compared to the control cells.

Thus, *Y. pestis* toxin *in vitro* inhibited the hormone-induced formation of PIM products and prostacycline in cell cultures of human umbilical vein endothelium. Previously we also showed a decrease of prostacycline content in rat blood plasma during the early stages of the development of plague intoxication [2]. The data presented here suggest that disturbances in hemostasis appearing in plague intoxication, particularly in its early stages, stem from the effect of *Y.pestis* toxic substances on Ca-mobilizing signal pathway system of vascular endothelial cells.

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