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Histamine and bradykinin stimulate the phosphoinositide turnover in human umbilical vein endothelial cells via different G-proteins

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The G-proteins which regulate hormonal turnover of phosphoinositide (PI) in human umbilical vein endothelial cells have been investigated. A 40–41 kDa doublet present in the membranes of these cells was selectively ADP ribosylated by pertussis toxin (PTx), and this doublet was $G_{i\alpha 2}$ and $G_{i\alpha 3}$ according to immunoblotting with specific antisera. By contrast, a doublet of 24–26 kDa proteins in the same membrane preparations was ADP ribosylated by the C_3 component of botulinum toxin (BoTx). PTx-dependent ADP ribosylation blocked stimulation of PI turnover by histamine, but did not affect stimulation by bradykinin, whereas BoTx (C_2 + C_3 components) had the opposite effect. Thus two different groups of G-proteins may be involved in hormone-dependent stimulation of PI turnover in human umbilical vein endothelial cells.

Protein, G-; Phosphoinositide turnover; (Human umbilical vein endothelial cell)

1. INTRODUCTION

Interaction of Ca^{2+}-mobilizing hormones with membrane receptors leads to phospholipase C activation which is accompanied by hydrolysis of phosphatidylinositol bisphosphate and the consequent generation of inositolphosphates and diacylglycerol [1]. Regulatory GTP-binding proteins (G-proteins) appear to play an important role in coupling of hormonal receptors to phosholipase C. Guanine nucleotides affected the hormonal stimulation of phosphoinositide hydrolysis in membrane preparations [2]. PTx, which ADP ribosylates and uncouples G_i-proteins from receptors, blocked the hormone-dependent activation of PI turnover in several types of mammalian cells [3]. However, in some cells hormone-dependent hydrolyis of phosphoinositides is insensitive to PTx [4–6].

In HUVEC, PI turnover can be activated by a variety of stimuli including bradykinin and histamine [4,7]. Nothing is known about the effect of guanine nucleotides and bacterial toxins on histamine-dependent stimulation of PI turnover in HUVEC. Activation of bradykinin receptors (B_2-type) in bovine pulmonary artery endothelial cells and porcine aorta endothelial cells leads to activation of PI turnover [4], Ca^{2+} mobilization, and stimulation of thromboxane and prostacyclin synthesis [8]. In these cells bradykinin receptors are coupled to PI turnover via PTx-insensitive G-protein [4,5].

We discovered that histamine-dependent activation of PI turnover in HUVEC is sensitive to PTx, whereas bradykinin-dependent activation of PI turnover is insensitive to PTx. At the same time botulinum toxin (C_2 and C_3 components) blocks the bradykinin-dependent activation of PI turnover and does not affect the histamine-stimulated PI turnover in these cells. The effects of PTx and BoTx are accompanied by ADP ribosylation of different groups of G-proteins: 40–41 kDa$_{\alpha i}$-subunits and 24–26 kDa G-proteins, respectively. These data suggest that in HUVEC histamine and bradykinin receptors activate PI turnover via different G-proteins.

2. MATERIALS AND METHODS

HUVEC were isolated and cultivated as previously described [7]. HUVEC were seeded in 12-well dishes and prelabeled with [^{3}H]myoinositol (5 μCi/ml) for 48 h. The confluent monolayer was washed three times with medium 199 without serum and incubated for 10 min in the same medium containing 10 mM LiCl. Inositol phosphates were determined as earlier described [7].

HUVEC membranes were prepared according to [4]. ADP ribosylation of membrane G-proteins by PTx and BoTx C_3 was performed according to [4,17]. Proteins were separated by SDS-PAGE [9]. Gels were fixed, stained with Coomassie blue G-250, and subjected to autoradiography for 18 h. Immunoblotting was performed according to [10]. Isolation and purification of PTx were conducted as described [11]. The ammonium sulphate fraction of BoTx contain-

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Abbreviations: G-protein, guanine nucleotide binding protein; G_i, inhibitory G-protein; IP_3, inositol trisphosphate; IP, inositol monophosphate; PI, phosphoinositide; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); GDPβS, guanosine 5′-*O*-(3-thiodiphosphate); PTx, pertussis toxin; BoTx, botulinum toxin; HUVEC, human umbilical vein endothelial cells

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ing C_2 and C_3 components, was a generous gift of Drs Y.V. Vertiev and A.I. Ephimenko (Institute of Epidemiology and Microbiology AMS, USSR); the pure C_3 component of BoTx was a generous gift of Dr Aktories (Rudolf-Buchheim-Institute für Pharmakologie der Universität Giessen, FRG). Antisera against $G_{0\alpha}$, $G_{i\alpha 2}$, $G_{i\alpha 3}$ were a kind gift of Dr J. Robishaw (USA). Nucleotides were from Boehringer Mannheim, FRG. Other reagents were from Sigma, USA.

3. RESULTS AND DISCUSSION

Differences in time-courses of hormone-dependent accumulation of IP and IP_3 are typical for many cells [4–6]. Following the treatment of HUVEC with histamine (0.1 mM) within 2 min the amount of IP_3 was significantly higher than the basal level. After 5 min IP_3 level revealed a plateau and was 3-fold higher compared to the basal level; 30 min after the incubation with histamine IP_3 decreased to basal level (fig.1), whereas 120 min later IP concentration was still 3.5-fold higher than in control (fig.1). Bradykinin (0.1 μM) stimulated PI turnover to a lesser extent than histamine. Maximal bradykinin-dependent increase of IP_3 was observed after 5 min (fig.1). The level of IP was significantly higher than the basal level 120 min after the incubation with bradykinin (fig.1). We earlier observed the same kinetics of bradykinin-dependent activation of PI turnover in bovine pulmonary artery endothelial cells [4]. The essential distinction between time-courses of histamine- and bradykinin-dependent accumulation of inositol phosphates in HUVEC may point to differences in the extent of coupling of histamine and bradykinin receptors to phospholipase C or to different mechanisms of coupling between these receptors and phospholipase C.

An important matter, therefore, is the identification of the regulatory G-protein(s), which couples hormonal receptor to phospholipase C. In HUVEC membranes PTx-dependent ADP ribosylation revealed a doublet of 40–41 kDa α-subunits of G_i-protein (fig.2). Analysis of these subunits by immunoblotting revealed the presence of $G_{i\alpha 2}$ and $G_{i\alpha 3}$. G_0-protein was absent (fig.2). Previously we observed that incubation of intact bovine pulmonary artery endothelial cells with submillimolar

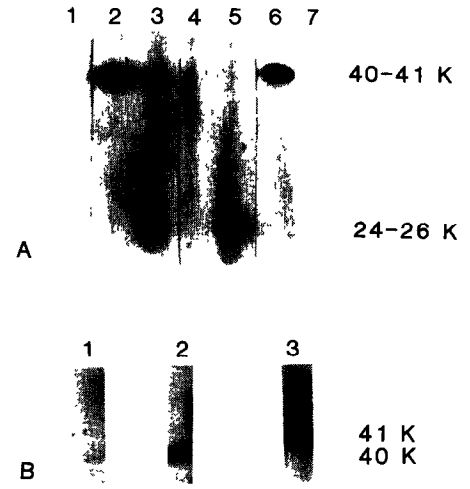

Fig.2. (A) PTx- and BoTx-dependent ADP ribosylations of G-proteins in HUVEC membranes. Membranes were prepared from HUVEC untreated or pretreated with 1 μg/ml of PTx or 1 μg/ml of BoTx (C_2+C_3) for 2 h and then incubated with [^{32}P]NAD in the presence or absence of preactivated PTx or BoTx (C_3) for 1 h. The reaction was stopped and the proteins were separated by polyacrylamide gel electrophoresis, and analyzed by autoradiography. (1) ADP ribosylation of untreated EC membranes without toxins; (2) Ptx-dependent ADP ribosylation of untreated EC membranes; (3) BoTx-dependent ADP ribosylation of untreated EC membranes; (4) PTx-dependent ADP ribosylation of EC preincubated with PTx; (5) BoTx-dependent ADP ribosylation of EC preincubated with PTx; (6) PTx-dependent ADP ribosylation of EC preincubated with BoTx; (7) BoTx-dependent ADP ribosylation of EC preincubated with BoTx. (B) Immunoblot of HUVEC membranes with anti-G_0, anti-G_{i2} and anti-G_{i3}. Membranes were loaded onto 12.5% polyacrylamide gels (100 μg per sample), resolved by SDS-PAGE and transferred to nitrocellulose. The blots were processed with indicated antisera and with peroxidase-conjugated goat antibodies to rabbit IgG. (1) antiserum A-10 ($G_{0\alpha}$ peptide antigen), 1 : 1000 dilution; (2) antiserum A-54 ($G_{i\alpha 2}$ peptide antigen), 1 : 1000 dilution; (3) antiserum A-56 ($G_{i\alpha 3}$ peptide antigen), 1 : 1000 dilution.

concentrations of GTPγS or GDPβS in medium 199 for 2 h leads to partial penetration of these guanine nucleotides [4]. The histamine- and bradykinin-dependent stimulation of PI turnover in HUVEC was potentiated after preincubation with GTPγS and inhibited after preincubation with GDPβS (fig.3). So we

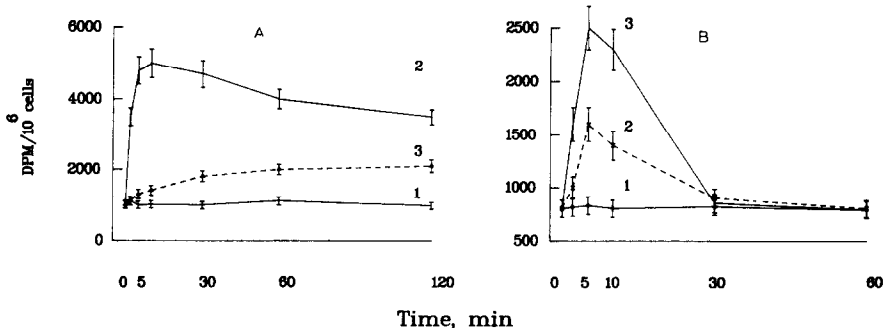

Fig.1. Time course for bradykinin- and histamine-stimulated IP (A) and IP_3 (B) production in HUVEC. myo[^{3}H]Inositol-prelabeled cells were incubated under standard conditions without addition (1 control) or with histamine (2, 0.1 mM) or bradykinin (3, 0.1 μM). The reaction was stopped after the times indicated. Each point represents the mean ± SD of triplicate determinations. The experiment shown is representative for 3 experiments.

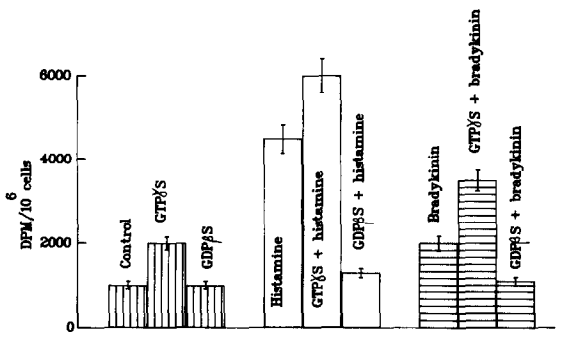

Fig.3. Effects of guanine nucleotides on histamine- and bradykinin-stimulated synthesis of IP in HUVEC. myo[^{3}H]Inositol-prelabeled cells were incubated under standard conditions in medium 199 without serum with 0.1 mM GTPγS or 0.1 mM GDPβS for 2 h, then the histamine (0.1 mM) or bradykinin (0.1 μM) were added for 10 min. Each bar represents mean ± SD of triplicate determinations. The experiment shown is representative for 5 experiments.

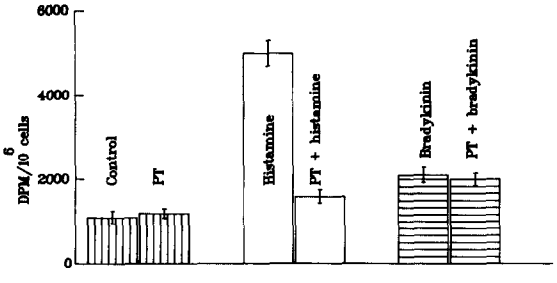

Fig.4. The effect of PTx on histamine- and bradykinin-stimulated synthesis of IP in HUVEC. myo[^{3}H]Inositol-prelabeled HUVEC were incubated under standard conditions in medium 199 without serum with PTx (1 μg/ml) for 2 h, then the histamine (0.1 mM) or bradykinin (0.1 μM) was added for 10 min. Each bar represents mean ± SD of triplicate determinations. The experiment shown is representative for 4 experiments.

concluded that G-protein(s) are involved in the action of these hormones.

It is well known that G-proteins involved in receptor-dependent regulation of phospholipase C can be distinguished by their different sensitivities to bacterial toxins. In neutrophils, macrophages, and foam cells hormone-dependent stimulation of phospholipase C occurs through PTx-sensitive G-protein [12,13]. In some cells the G-protein responsible for coupling is PTx-insensitive. This type of G-protein was found in hepatocytes, stimulated by vasopressin and angiotensin II; in 3T3 fibroblasts, stimulated by thrombin, and in porcine and bovine endothelial cells, stimulated by bradykinin [4,6,14–16]. Figs. 4 and 5 show that after 2 h incubation with PTx (1 μg/ml) the histamine-dependent stimulation of PI turnover was blocked, whereas the bradykinin-dependent stimulation of PI turnover was unchanged. At the same time after 2 h of incubation of cells with BoTx (1 μg/ml, C_2+C_3 components) the bradykinin-dependent stimulation of PI turnover was blocked, but the histamine-dependent stimulation of PI turnover was not affected. The inhibitory effect of bacterial toxins on PI turnover was accompanied by ADP ribosylation of the corresponding G-proteins: 2 h incubation of HUVEC with C_2+C_3 components of BoTx blocked the ^{32}P-labeled C_3-dependent ADP ribosylation of 24–26 kDa G-proteins in membranes isolated from these cells, whereas 2 h incubation of HUVEC with PTx abolished the PTx-dependent [^{32}P]ADP ribosylation of 40–41 kDa α_i-subunits in the membranes isolated from the pretreated cells (fig.2).

It has been established, that the C_3 component of BoTx ADP ribosylates the family of GTP-binding ϱ-proteins with low molecular mass of 24–26 kDa [17]. Functions of ϱ-G-proteins are currently unknown. The C_2 component of BoTx ADP ribosylated non-muscle actin, and induced depolymerization of actin filaments in the cells [17]. Therefore, the inhibition by BoTx of the bradykinin-dependent stimulation of PI turnover in HUVEC may depend both on ADP ribosylation of low molecular mass G-proteins, and on actin depolymerization. Actin depolymerization can affect the bradykinin-dependent stimulation of PI turnover if bradykinin-stimulated phospholipase C is associated with actin filaments. Our results about the regulation of the bradykinin-dependent activation of PI turnover in HUVEC and other endothelial cells by guanine nucleotides [4] support the suggestion concerning the involvement of 24–26 kDa G-proteins in this process.

Thus, in a homogeneous population of human umbilical vein endothelial cells histamine and bradykinin receptors are coupled to phospholipase C via different G-proteins. Low molecular mass G-proteins, with α-subunits of 24–26 kDa, ADP ribosylated by the C_3 component of BoTx, may participate in coupling of bradykinin receptor to phospholipase C. G_i-proteins with α-subunits of 40–41 kDa, ADP ribosylated by PTx, couple the histamine receptor to the same enzyme or to another isoform of phospholipase C in HUVEC.

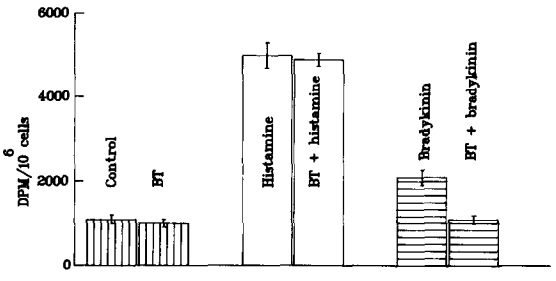

Fig.5. The effect of BoTx on histamine- and bradykinin-stimulated synthesis of IP in HUVEC. myo[^{3}H]Inositol-prelabeled HUVEC were incubated under standard conditions in medium 199 without serum with BoTx (C_2+C_3, 1 μg/ml) for 2 h, then the histamine (0.1 mM) or bradykinin (0.1 μM) were added for 10 min. Each bar represents mean ± SD of triplicate determinations. The experiment shown is representative for 6 experiments.

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