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Flow-induced prostacyclin production is mediated by a pertussis toxin-sensitive G protein

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Fluid flow and several other agonists induce prostacyclin (PGI_2) production in endothelial cells. G proteins mediate the response of a large number of hormones such as histamine, but the transduction pathway of the flow signal is unclear. We found that GDPβS and pertussis toxin inhibited flow-induced prostacyclin production in human umbilical vein endothelial cells. In addition, flow potentiated the histamine-induced production of PGI_2. This suggests that flow stimulates prostacyclin production via a pertussis toxin-sensitive G protein and modulates the stimulus-response coupling of other agonists.

Prostacyclin; G protein; Pertussis toxin; Shear stress; Histamine; Endothelial cell

1. INTRODUCTION

Endothelial cells produce prostacyclin (PGI_2) when stimulated by fluid flow and agonists such as ATP, bradykinin, histamine and leukotrienes [1–4]. These hormones activate phospholipid turnover, leading to the liberation of the cyclooxygenase substrate, arachidonic acid, which is considered to be a rate-limiting step in prostaglandin synthesis [5].

Agonist-induced phospholipase activation is mediated by G proteins [6,7], suggesting that PGI_2 synthesis depends on G protein activation. Accordingly, direct stimulation of G proteins by AlF_4^- and GTPγS leads to PGI_2 production in human umbilical vein endothelial cells (HUVECs) [8]. In bovine endothelial cells, pertussis toxin (PTX) inhibits the stimulation of PGI_2 release by leukotrienes C_4 and D_4 [9], but potentiates that induced by ATP [10].

Fluid flow stimulates endothelial phospholipid turnover [11,12], suggesting that flow triggers a pathway leading to PGI_2 synthesis which probably involves phospholipase activation, however, earlier events in the flow signal transduction are unclear. It was recently hypothesized that, in the presence of exogeneous ATP, flow could increase ATP levels near the cell membrane by overcoming local degradation by ectonucleotidases, thereby stimulating the cells [13–16].

We found that a PTX-sensitive G protein mediates the flow-induced PGI_2 synthesis in HUVECs. In addition, we show that flow can potentiate the effect of histamine, which, unlike ATP, is not rapidly hydrolyzed at the membrane surface. This suggests that flow may affect the stimulus-response coupling of other agonists in HUVECs by an intracellular mechanism.

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2. MATERIALS AND METHODS

ATP-free Medium 199 was from Cell Culture Laboratories (Cleveland, OH) and fetal calf serum (FCS) from Hyclone (Logan, VT). Collagenase (type A) was from Boehringer-Mannheim (Indianapolis, IN). 6-*keto*-Prostaglandin $F_{1\alpha}$ (6-*keto*-$PGF_{1\alpha}$) and PTX were from Calbiochem (San Diego, CA) and 6-[5,8,9,11,12,14,15-^{3}H(N)]*keto*-$PGF1\alpha$ (150 Ci/mmol) was from NEN (Boston, MA). IgGSorb was from The Enzyme Center (Malden, MA). All other chemicals including 6-*keto*-$PGF_{1\alpha}$ antiserum were from Sigma (St. Louis, MO).

HUVECs were cultured as described previously [2]. Confluent cells were pre-incubated in Medium 199 without ATP with 2 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin with no drug added for 2 h, or with 0.2 μg/ml PTX for 2 h, or with 0.2 mM guanine nucleotides for 6 h. They were then subjected to a steady shear stress of 25 dynes/cm^2 in a parallel-plate flow chamber (described in [2]) for up to 6 h, which was perfused with fresh pre-incubation medium plus 20% FCS and half of the drug concentration used in the pre-incubation. Static controls were kept in Petri dishes. ATP levels in the medium were <7.5 nM, as determined by an assay based on the luciferin-luciferase reaction (Sigma, St. Louis, MO). At the end of some experiments, histamine was added to a final concentration of 10 μM and the medium was sampled after 15 min. To study the synergy of flow and histamine, histamine was added to a final concentration of 10 μM 20 min after the onset of shear and while the cells were still subjected to flow; samples were taken for 6 h thereafter. Samples were stored at −20°C.

Medium PGI_2 levels were determined by radioimmunoassay of its stable metabolite 6-*keto*-$PGF_{1\alpha}$. Overall production rates were obtained by linear regression of each entire time-course of PGI_2 accumulation and compared by a two-tailed *t*-test for statistical significance [17]. For experiments with histamine, initial production rates were obtained by linear regression on data points from 0 to 5 min and compared as previously.

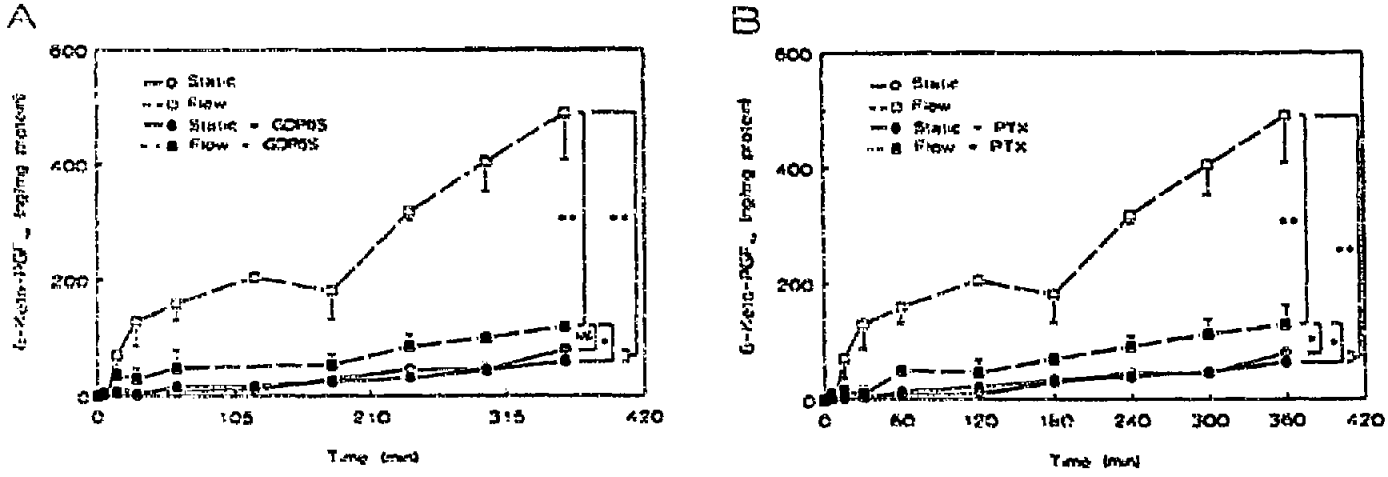

Fig. 1. Effect of G protein inhibitors on flow-induced release of 6-*keto*-$PGF_{1\alpha}$. Release of 6-*keto*-$PGF_{1\alpha}$ by HUVECs under static conditions and subjected to a shear stress of 25 dy/cm². Cells were pre-incubated with 0.2 mM GDPβS (A) or 0.2 μg/ml PTX (B). Error bars represent the S.E.M. (n = 2). Overall production rates of 6-*keto*-$PGF_{1\alpha}$ are compared; $^{*}P < 0.05$; $^{**}P < 0.01$; NS, not significant.

3. RESULTS AND DISCUSSION

Fluid flow induced a burst in PGI_2 release followed by a period with little net production at 1–3 h, and a later phase where a steady rate of synthesis was observed (Fig. 1), which is in agreement with published observations [1,2]. GDPβS and PTX inhibited both the initial burst and the later phase of stimulation by flow. The production of PGI_2 by static cells was unaffected by GDPβS or PTX. These results suggest for the first time that a PTX toxin-sensitive G protein mediates a flow-induced response in HUVECs.

The general activator of G proteins, GTPγS, stimulated PGI_2 production to a lesser degree than flow (Fig. 2A). Addition of 10 μM histamine led to an additional release of PGI_2 which was similar for static and sheared cells, but approximately 50% less for cells treated with GTPγS (Fig. 2B). The effect of GTPγS is consistent with a functional pathway linking G protein activation to PGI_2 synthesis in our cells. The relatively low production rate of PGI_2 in GTPγS-treated cells may be due to insufficient agonist loading into the cells, or due to the stimulation of inhibitory G proteins by GTPγS [8].

Flow and ATP synergistically increase intracellular calcium levels (Ca_i) [13–16]. Some authors have claimed that flow alone may not increase Ca_i [14,15], an event most likely required for flow-induced PGI_2 synthesis

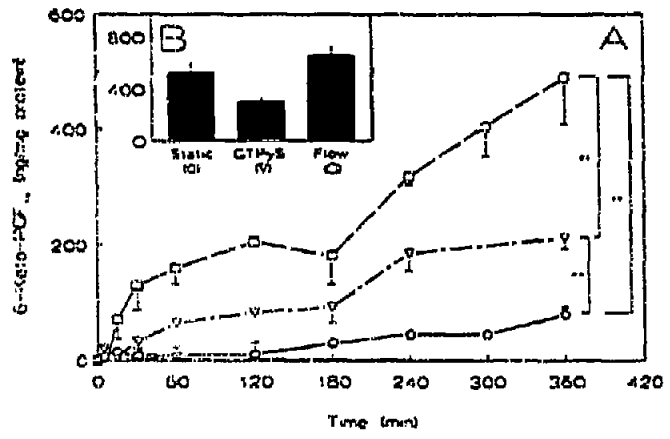

Fig. 2. Effect of flow and GTPγS on the release of 6-*keto*-$PGF_{1\alpha}$. (A) Release of 6-*keto*-$PGF_{1\alpha}$ by static and flow controls (□), or under static conditions for HUVECs pre-incubated with 0.2 mM GTPγS (▽). Error bars represent the S.E.M. (n = 2). Overall production rates of 6-*keto*-$PGF_{1\alpha}$ are compared; $^{**}P < 0.01$. (B) Additional production of 6-*keto*-$PGF_{1\alpha}$ due to the addition of 10 μM histamine to the medium at the end of the experiment shown in A.

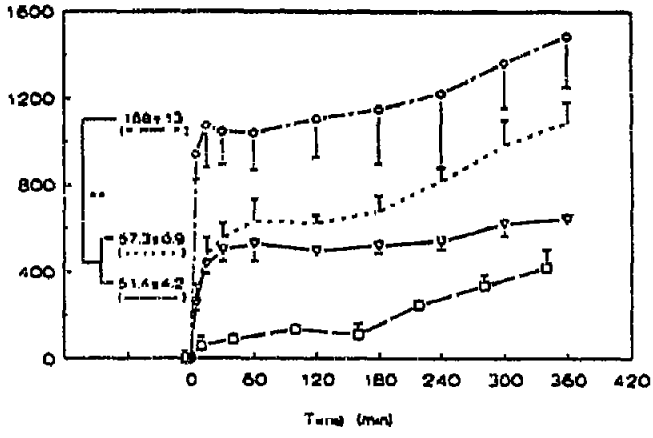

Fig. 3. Synergism of flow- and histamine-induced release of 6-*keto*-$PGF_{1\alpha}$. Release of 6-*keto*-$PGF_{1\alpha}$ by HUVECs subjected to flow (data shown is the accumulation of 6-*keto*-$PGF_{1\alpha}$ starting 20 min after the onset of shear (□), by HUVECs stimulated with 10 μM histamine under static conditions (▽), and stimulated with 10 μM histamine under flow conditions after pre-shearing for 20 min (▽). The sum of the productions due to histamine alone and flow alone is also shown (– – –). Error bars represent the S.E.M. (n = 2). Initial production rates of 6-*keto*-$PGF_{1\alpha}$ are compared; $^{**}P < 0.01$.

[12]. The ATP levels in the medium used in this study were very low (<7.5 nM) and others also observed that flow induces PGI_2 synthesis in ATP-free medium [3]. Furthermore, PTX has been shown to potentiate the ATP-induced PGI_2 production [10], thus our results disagree with an ATP-mediated mechanism of flow-induced PGI_2 synthesis.

Addition of 10 μM histamine under flow conditions resulted in an initial production rate of PGI_2 by HUVECs greater than the sum of the individual rates due to flow and histamine (Fig. 3), which suggests that flow also synergizes with histamine. Unlike ATP, histamine is not rapidly degraded at the cell membrane, thus it is doubtful that facilitated mass transport by flow increased the agonist concentration at the cell surface. We postulate that flow can modulate the stimulus–response coupling of other agonists in HUVECs via an intracellular mechanism.

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