

# Autocrine regulation of endothelial exocytosis: von Willebrand factor release is induced by prostacyclin in cultured endothelial cells

Ulrich M. Vischer<sup>a,\*</sup>, Ursula Lang<sup>b</sup>, Claes B. Wollheim<sup>a</sup>

<sup>a</sup>Division of Clinical Biochemistry, Department of Medicine, Geneva University Hospital, 1 rue Michel Servet, 1211 Geneva 4, Switzerland

<sup>b</sup>Division of Endocrinology, Department of Medicine, Geneva University Hospital, 1211 Geneva, Switzerland

Received 30 January 1998

**Abstract** Vascular endothelial cells respond to external stimuli by altering the secretion of several bioactive molecules, including von Willebrand factor (vWf), prostacyclin (PGI<sub>2</sub>) and nitric oxide (NO). The release of all three molecules is regulated by a rise in cytosolic calcium ([Ca<sup>2+</sup>]<sub>i</sub>). In the present study we investigated whether cAMP-dependent signaling provides differential regulation of these effector systems by modulating the effect of [Ca<sup>2+</sup>]<sub>i</sub> in cultured human endothelial cells. The stable PGI<sub>2</sub> analog iloprost, like other cAMP-raising agents (forskolin and adenosine), caused an acute dose-dependent increase in vWf release and potentiated the secretory response to thrombin. In contrast, iloprost, forskolin and adenosine failed to induce PGI<sub>2</sub> release and inhibit thrombin-induced release. Our findings indicate cAMP-raising agents have opposite effects on [Ca<sup>2+</sup>]<sub>i</sub>-mediated vWf secretion and PGI<sub>2</sub> release. PGI<sub>2</sub> may potentiate vWf release and inhibit its own release in an autocrine manner.

© 1998 Federation of European Biochemical Societies.

**Key words:** Von Willebrand factor; Prostacyclin; Endothelial cell; Nitric oxide

## 1. Introduction

Vascular endothelial cells respond to external stimuli by altering the secretion of several bioactive molecules, including von Willebrand factor (vWf), prostacyclin (PGI<sub>2</sub>) and nitric oxide (NO). vWf is released by exocytosis from specialized secretory granules called Weibel-Palade bodies [1]. Exocytosis in response to thrombin and several other agonists is mediated by a rise in cytosolic calcium ([Ca<sup>2+</sup>]<sub>i</sub>) and activation of a calmodulin-dependent signaling pathway [2]. vWf release can also be induced by phorbol esters, suggesting a role for protein kinase C (PKC). However, thrombin-induced vWf release is not prevented by PKC inhibitors [3]. Thus, a rise in [Ca<sup>2+</sup>]<sub>i</sub> is thought to be the main signaling event for exocytosis. Prostacyclin production, via the activation of phospholipase A<sub>2</sub>, is also a calcium-dependent process. In addition it is modulated by protein tyrosine kinases and possibly by PKC [4,5]. NO synthesis is also driven by a rise in [Ca<sup>2+</sup>]<sub>i</sub>. Calcium/calmodulin directly binds endothelial nitric oxide synthase (eNOS) and stimulates NO synthesis in the vicinity of the plasma membrane, where eNOS is associated with discrete membrane structures called caveolae [6]. Thus, a rise in [Ca<sup>2+</sup>]<sub>i</sub> is a key signaling event for three different effector systems in a single cell type. Given the different physiological functions of these effector systems, it is very likely that they are regulated in a differential manner. Such differential regulation could be accounted for in part by different subcellular localization of the

calcium signal or by specialization of endothelial cells in different vascular beds. However, additional signaling pathways may provide differential regulation by modulating the effect of [Ca<sup>2+</sup>]<sub>i</sub>.

We have recently observed that vWf secretion is also regulated by cAMP. Pharmacological agents such as forskolin and 8-bromo-cAMP can induce vWf secretion, and strongly potentiate the secretory response to thrombin [7]. Furthermore, the receptor-mediated agonists epinephrine and adenosine potentiate the response to [Ca<sup>2+</sup>]<sub>i</sub>-raising agents such as thrombin and ATP, in a cAMP-dependent manner [7,8]. Prostacyclin produced by endothelial cells acts via a specific, adenylate cyclase-coupled receptor [9]. Prostacyclin is thought to regulate endothelial permeability via a cAMP-dependent signaling pathway, suggesting a role for prostacyclin in the autocrine regulation of endothelial function [10]. In the present study we investigated the effect of prostacyclin, using its stable analog iloprost, on vWf secretion and on prostacyclin production in cultured human endothelial cells. In addition, since endothelial NO is also an autocrine regulator of endothelial function, we tested the effect of NO on vWf secretion.

## 2. Materials and methods

### 2.1. Materials

RPMI 1640 was from Gibco BRL (Gaithersburg, MD, USA), fetal calf serum (FCS) and collagenase were from Seromed (Berlin, Germany). Endothelial cell growth supplement (ECGS) was from Upstate Biotechnology Inc. (Lake Placid, NY, USA). Anti-vWf antibodies were from Dako (Glostrup, Denmark). Iloprost was from Shering (Berlin, Germany). Adenosine was from Fluka (Buchs, Switzerland). Linsidomine (SIN-1) and sodium nitroprusside were from RBI (Natick, MA, USA). Human thrombin, 3-isobutyl-1-methyl-xanthine (IBMX) and forskolin were from Sigma (St. Louis, MO, USA).

### 2.2. Cell culture

Primary cultures of endothelial cells (HUVECs) were obtained from individual human umbilical veins by collagenase digestion as described previously [11]. They were grown in medium RPMI 1640 supplemented with 10% FCS, 90 µg/ml heparin and 15 µg/ml ECGS. Cells were used during passages 1 or 2. Tissue culture dishes as well as the multiwell plates (Costar, Cambridge, MA, USA) were coated with 0.1% gelatin.

### 2.3. vWf release studies

Confluent monolayers of HUVECs grown in 24-well dishes were washed three times and preincubated in Krebs-Ringer-bicarbonate buffer (KRBH, 120 mmol/l NaCl, 4.75 mmol/l KCl, 1.2 mmol/l KH<sub>2</sub>PO<sub>4</sub>, 0.6 mmol/l MgSO<sub>4</sub>, 1.2 mmol/l CaCl<sub>2</sub>, 25 mmol/l NaHCO<sub>3</sub>, 25 mmol/l HEPES, pH 7.4) supplemented with 0.1% BSA, and preincubated for 5–10 min at 37°C. After a fourth wash, cells were incubated in KRBH with the different agents. The collected supernatants were cleared of cell debris by centrifugation (16 000 × g for 60 s) and stored at –20°C for later assay. All pharmacological agents were directly dissolved in the incubation medium, only forskolin and IBMX were dissolved in DMSO. The final concentration of DMSO in the incubation medium did not exceed 0.2%, a concentration which

\*Corresponding author. Fax: (41) (22) 702 55 43.  
E-mail: ulrich.vischer@medecine.unige.ch

has no effect on vWf release. vWf was measured in the supernatants by ELISA as described previously [12]. A standard curve was constructed from serial dilutions of normal pooled plasma, assuming a plasma concentration of 10  $\mu\text{g/ml}$ . Results are usually expressed in ng/well/time unit.

#### 2.4. Prostacyclin release studies

Confluent HUVECs grown in 12-well dishes were washed three times in KRBH and incubated at 37°C in 1.2 ml KRBH. After 10 min, 0.6 ml was gently removed. The various agonists were then added with minimal shaking. The incubation was continued for the indicated times, and the supernatants were collected for later assays. For both vWf and PGI<sub>2</sub>, release was calculated as the difference in content between the two successive samples. This procedure was used to minimize the possible effect of mechanical stress (due to cell handling) on PGI<sub>2</sub> release.

vWf was measured by ELISA. PGI<sub>2</sub> was measured by a specific radioimmunoassay of its stable metabolite 6-keto-PGF<sub>1 $\alpha$</sub>  as previously described [13]. The agonists tested, in particular iloprost, did not interfere with the immune reaction when added to standard samples. We observed considerable variation in basal PGI<sub>2</sub> release between cell batches. The results were therefore expressed in relative values, i.e. as a percentage of release from cells stimulated with thrombin (0.5 U/ml) from the same cell preparation. Unless indicated otherwise, results are shown as mean  $\pm$  S.E.M. Statistical analysis was done using the two-tailed, paired Student's *t*-test.

### 3. Results

#### 3.1. Effect of iloprost on vWf release from cultured HUVECs

To investigate the effect of prostacyclin on vWf release we employed the stable analog iloprost on vWf release from cultured HUVECs (Fig. 1). Iloprost (1 nmol/l to 1  $\mu\text{mol/l}$ ) added

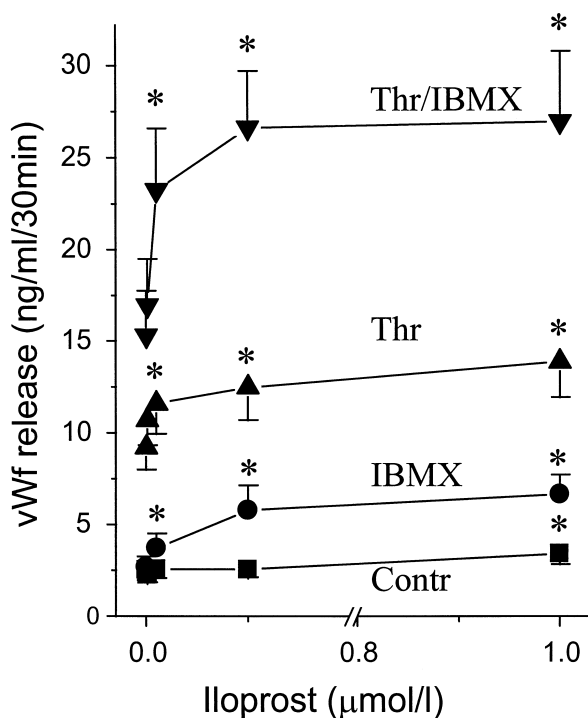


Fig. 1. Effect of iloprost on vWf release: dose response. Confluent HUVECs grown in 24-well plates were incubated for 30 min at 37°C with increasing concentrations of iloprost (0.001–1  $\mu\text{mol/l}$ ) added alone (squares), or together with 100  $\mu\text{mol/l}$  IBMX (circles), 0.5 U/ml thrombin (upwards triangles) or IBMX and thrombin combined (downwards triangles). vWf release was measured in the supernatant by ELISA. Results are shown as the mean  $\pm$  S.E.M. of five experiments. \**P* < 0.05 compared to unstimulated control cells.

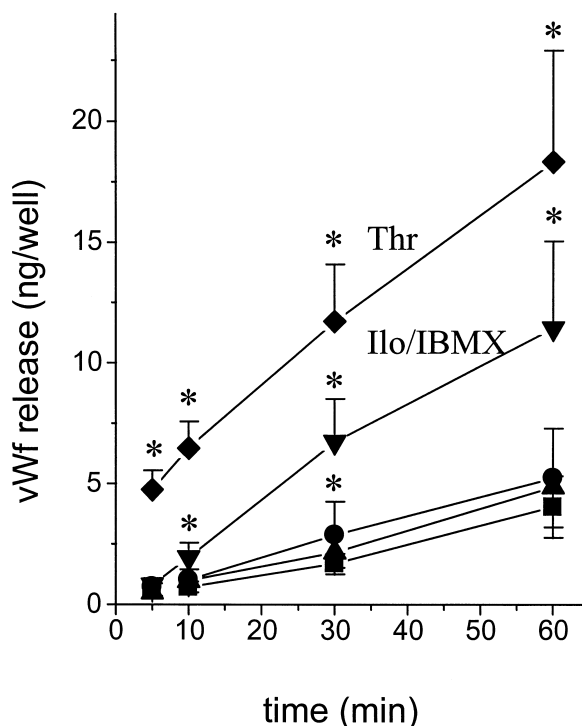


Fig. 2. Iloprost-induced vWf release: time course. Confluent HUVECs grown in 24-well plates were incubated for the indicated times with 1  $\mu\text{mol/l}$  iloprost, alone (circles) or together with 100  $\mu\text{mol/l}$  IBMX (downwards triangles), or 0.5 U/ml thrombin (diamonds). Control wells were incubated with KRBH alone (squares) or 100  $\mu\text{mol/l}$  IBMX (upwards triangles). vWf release was measured in the supernatant by ELISA. Results are the mean  $\pm$  S.E.M. of five experiments. \**P* < 0.05 compared to unstimulated control cells.

for 30 min caused a 1.4-fold increase (from  $2.5 \pm 0.6$  to  $3.4 \pm 0.6$  ng/well/30 min, *P* = 0.04, *n* = 5) at the highest concentration. Addition of IBMX alone (100  $\mu\text{mol/l}$ ), which blocks cAMP degradation by inhibiting nucleotide phosphodiesterases, had no effect on vWf release. However, when added together with IBMX, the secretory response to iloprost was clearly potentiated. The maximal response (at 1  $\mu\text{mol/l}$ ) was a 2.5-fold increase in vWf release (from  $2.7 \pm 0.6$  to  $6.7 \pm 1.1$  ng/well/30 min, *P* = 0.02), and there was a shift to the left in the dose-response curve, with a secretory response detectable at concentrations of iloprost as low as 10 nmol/l (*P* = 0.01, *n* = 5). We also observed a potentiating effect of iloprost on thrombin-induced vWf release. Indeed, the secretory response to thrombin and iloprost added together was greater than the sum of the responses to either agent added alone. Iloprost (1  $\mu\text{mol/l}$ ) caused an increase in vWf release from  $9.2 \pm 1.2$  to  $13.9 \pm 2.0$  ng/well/30 min (*P* = 0.01) when added to thrombin and from  $15.3 \pm 2.5$  to  $27.0 \pm 3.8$  ng/well/30 min (*P* = 0.01) when added to thrombin and IBMX.

We next performed a comparative time course study of iloprost- and thrombin-induced vWf secretion (Fig. 2). The response to thrombin was rapid, reaching significance already after 5 min, the earliest time point studied. In contrast, iloprost-induced vWf release could be demonstrated only after 10 and 30 min (*P* < 0.05 for both time points). The addition of IBMX potentiated the response to iloprost but failed to alter the time course: a significant but small response was observed after 10 min, and became more obvious only at later time points. This delayed secretory response to iloprost is highly

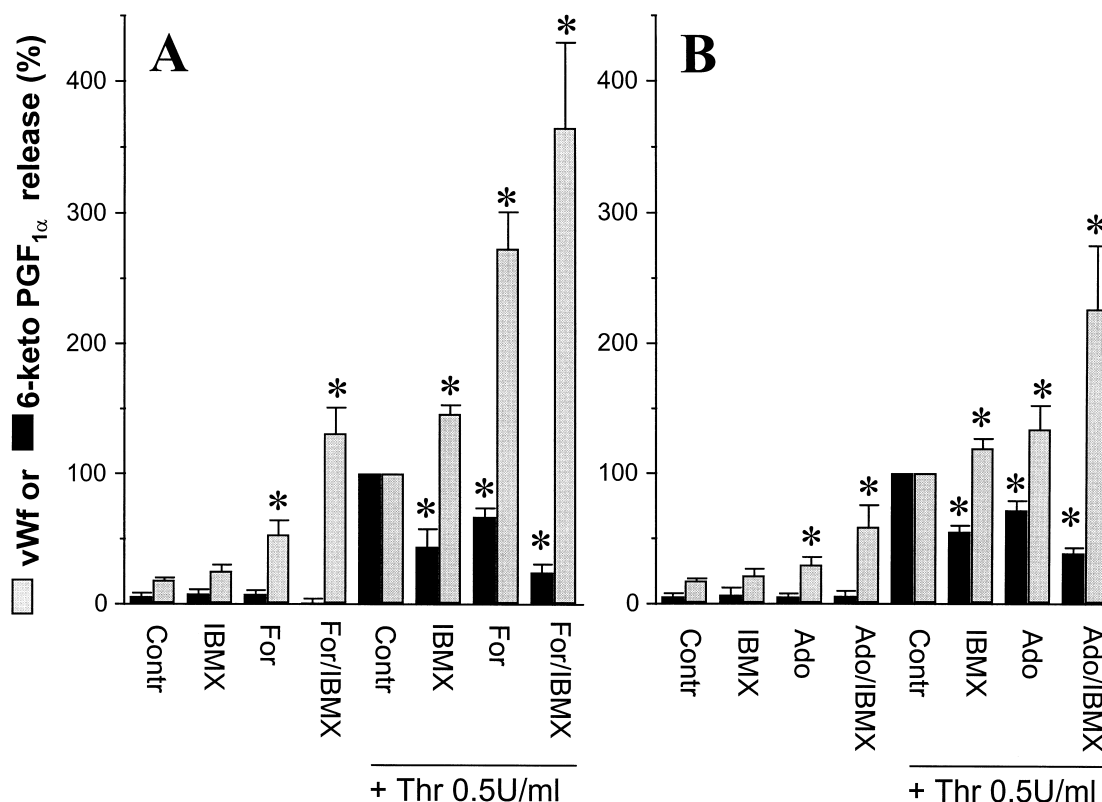


Fig. 3. Differential regulation of vWf secretion and PGI<sub>2</sub> release by cAMP-raising agents. Confluent HUVECS grown in 12-well plates were incubated at 37°C in 1.2 ml KRBH. After 10 min, a first 0.6 ml sample was gently removed. IBMX (100 μmol/l), forskolin (For, 10 μmol/l), adenosine (Ado, 100 μmol/l) and thrombin (Thr, 0.5 U/ml) were then added as indicated. The incubation was continued for 20 min and the supernatants collected for vWf and PGI<sub>2</sub> assay. For both parameters, release was calculated as the difference in content between the two successive samples, and expressed as a percentage of the response to thrombin alone. Results with forskolin (A) and adenosine (B) derive from two distinct sets of experiments. Results are the mean ± S.E.M. of five experiments. \**P* < 0.05 compared to unstimulated control cells.

reminiscent of our previous studies with other cAMP-raising agents such as forskolin and adenosine.

Thrombin has been reported to induce PGI<sub>2</sub> release from cultured endothelial cells. We therefore wondered whether PGI<sub>2</sub> released into the incubation buffer could mediate in part the secretory response to thrombin. However, thrombin-induced vWf release was not affected by preincubation with the cyclooxygenase inhibitor indomethacin (1–10 μmol/l, added for 10–40 min) (not shown).

### 3.2. Effect of cAMP-raising agents on vWf secretion and PGI<sub>2</sub> release

To compare the effect of cAMP-raising agents on vWf secretion and PGI<sub>2</sub> release, we optimized the incubation conditions to measure both secretory products from the same cell preparation. HUVECs were preincubated for 10 min in 1.2 ml KRBH. After removal of 0.6 ml incubation buffer, the cAMP-raising agents and thrombin were added for 20 min (Fig. 3). As previously reported [7], forskolin (± IBMX) induced vWf release, and potentiated the secretory response to thrombin (Fig. 3A). In contrast, PGI<sub>2</sub> release was not reliably detectable in unstimulated cells, and was not stimulated by forskolin. Thrombin-induced PGI<sub>2</sub> release was inhibited by both 100 μmol/l IBMX (−54 ± 14%, *P* = 0.01, *n* = 5) and 10 μmol/l forskolin (−33 ± 7%, *P* = 0.01, *n* = 5). Addition of the two agents combined inhibited thrombin-induced PGI<sub>2</sub> release by 76 ± 6% (*P* < 0.01, *n* = 5). These findings strongly suggest that increased cellular cAMP inhibits thrombin-induced

PGI<sub>2</sub> release. We also attempted to test the effect of the cAMP analog 8-bromo-cAMP, but unexpectedly this compound interfered with the PGI<sub>2</sub> assay. Since adenosine induces vWf release in a cAMP-dependent mechanism [8], we compared the effect of adenosine on vWf and PGI<sub>2</sub> release (Fig. 3B). Adenosine both in the absence or presence of IBMX induced vWf release and potentiated the response to thrombin, in agreement with our earlier report [8]. Similar to the pattern seen with forskolin, adenosine failed to induce PGI<sub>2</sub> release, and inhibited thrombin-induced PGI<sub>2</sub> release by 29 ± 7% (*P* = 0.03, *n* = 4) when added alone and by 62 ± 4% (*P* = 0.001, *n* = 4) when added together with IBMX.

### 3.3. Effect of iloprost on thrombin-induced PGI<sub>2</sub> release

The present data with forskolin, IBMX and adenosine indicate that thrombin-induced PGI<sub>2</sub> release can be inhibited by a rise in cellular cAMP content. Since PGI<sub>2</sub> itself is a cAMP-raising agent, our findings raise the possibility that PGI<sub>2</sub> inhibits its own release in an autocrine manner. To test this hypothesis, we compared the effect of iloprost on vWf secretion and PGI<sub>2</sub> release (Fig. 4). Iloprost or IBMX was added for 20–40 min prior to a 10 min stimulation with thrombin. Iloprost, alone or in the presence of IBMX, again caused vWf secretion and potentiated thrombin-induced vWf release. In contrast, iloprost caused a 35 ± 9% inhibition of thrombin-induced PGI<sub>2</sub> release (*P* = 0.02, *n* = 5). The inhibitory effect of IBMX was even stronger than in the previous experiments (with a 71 ± 7% inhibition), likely due to the longer preincu-

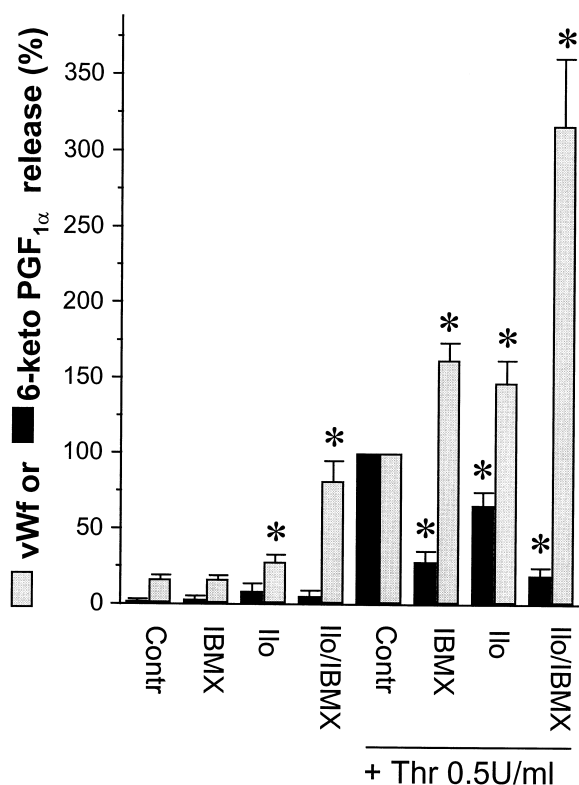


Fig. 4. Effect of iloprost on vWf secretion and PGI<sub>2</sub> release. Confluent HUVECs grown in 12-well plates were incubated at 37°C in 1.2 ml KRBH. After 10 min, a first 0.6 ml sample was gently removed. IBMX (100 µmol/l) and iloprost (Ilo, 1 µmol/l) were then added as indicated. The incubation was continued for 30–50 min, and thrombin was added for the last 10 min. vWf and PGI<sub>2</sub> release into the supernatant were measured by ELISA. For both parameters, release was calculated as the difference in content between the two successive samples, and expressed as a percentage of the response to thrombin alone. Results are the mean ± S.E.M. of five experiments. \**P* < 0.05 compared to unstimulated control cells.

bation time. However, the inhibitory effect of iloprost and IBMX combined was still even more pronounced ( $81 \pm 5\%$  inhibition), reaching statistical significance when compared to both thrombin alone or to thrombin/IBMX (*P* < 0.05, *n* = 5).

### 3.4. Effect of NO donors on thrombin-induced vWf release

We investigated whether activation of a cGMP-dependent pathway could modulate vWf release in cultured HUVECs. We tested two NO donors, linsidomine (SIN-1) and sodium nitroprusside (SNP). Both agents failed to influence basal or thrombin-stimulated vWf release (Fig. 5). Similar negative results were obtained using the cell-permeant cGMP analogs 8-bromo-cGMP or Sp-8-CPT-cGMPS.

## 4. Discussion

Our earlier studies have demonstrated that a cAMP-dependent signaling pathway is involved in the control of regulated vWf release from cultured HUVECs. Forskolin, an activator of adenylate cyclase, and the cAMP analog 8-bromo-cAMP both induce vWf release and potentiate the secretory response to thrombin [7]. These responses are potentiated by co-incubation with IBMX, an inhibitor of phosphodiesterases

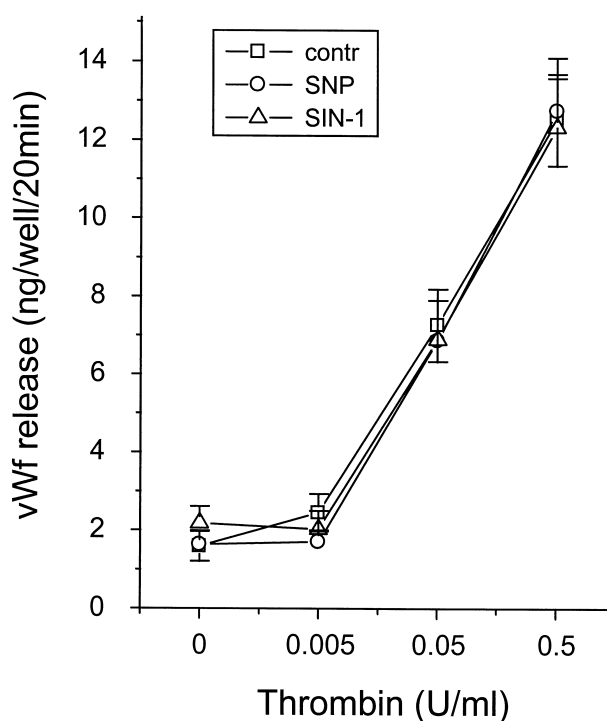


Fig. 5. Effect of NO donors on vWf release. Confluent HUVECs grown in 24-well plates were incubated for 30 min at 37°C in the presence of 1 mM sodium nitroprusside (SNP) or 1 mM linsidomine (SIN-1). Thrombin at various concentrations was added for the last 10 min (four experiments) or for the whole 30 min incubation (two experiments). Given the lack of differences the results of the six experiments were pooled for calculations, and are shown as mean ± S.E.M.

which blocks cAMP degradation. Similar responses were observed with receptor-mediated cAMP-raising agents such as epinephrine and adenosine [7,8]. The effect of cAMP-raising agents is due to regulated release from Weibel-Palade bodies, as indicated by its rapid time course (< 30 min). Furthermore, vWf secreted in response to forskolin consists of high molecular weight multimers, typical of regulated release (U.M. Vischer, unpublished observations). Although the secretory responses we observed with receptor-mediated agonists (epinephrine, adenosine) were small, cAMP-dependent vWf secretion is likely to be of physiological significance. Indeed, in humans epinephrine infusion causes a rise in circulating vWf levels [14]. Further, the rise in plasma vWf levels associated with physical activity in healthy subjects is inhibited by the β-adrenoceptor blocker propranolol [15].

The main finding of the present study is that iloprost, a prostacyclin agonist known to increase cellular cAMP content [9], also induced vWf secretion in cultured HUVECs, either when added alone or in combination with IBMX. Moreover, iloprost potentiated the secretory response to thrombin. The secretory response to iloprost is slower than to thrombin, but still occurred within 30 min, indicating secretion from a pre-formed store rather induction of vWf synthesis and constitutive release. From a physiological point of view, it is unexpected that cAMP-mediated signaling activated by adenosine and prostacyclin stimulates vWf secretion in the endothelium, while it inhibits platelet activation (and by inference vWf release from α-granules). However, exocytosis evoked by cAMP (alone and/or in synergism with intracellular calcium) is not

surprising since it has been observed in many secretory cell systems, such as insulin-secreting  $\beta$ -cells [16], the exocrine pancreas [17] and others. Our observations are compatible with rat hindlimb perfusion studies showing a potentiating effect of forskolin on vWf secretion induced by the calcium-mobilizing agents bradykinin and platelet-activating factor [18].

Prostacyclin infusion is used in the treatment of Raynaud's disease [19] and of primary pulmonary hypertension (PPH) [20]. In the case of PPH, treatment is associated with a marked decrease in vWf levels, as well as a decrease in other endothelial markers such as t-PA and PAI-1 [20,21]. The present data suggest that prostacyclin does not decrease vWf levels by a direct effect on endothelial vWf release. The effect of prostacyclin is more likely to be due to an indirect effect, such as improved vessel perfusion secondary to its hemodynamic effect, resulting in decreased shear stress [22]. An direct inhibitory effect on platelet vWf release is also a possibility.

vWf secretion, PGI<sub>2</sub> and NO release are all induced by an increase in  $[Ca^{2+}]_i$ , raising the issue of the differential regulation of these effector systems. Our findings demonstrate that while cAMP-mediated signaling induces vWf release, it inhibits PGI<sub>2</sub> release from cultured HUVECs. This opposite effect was observed in response to forskolin, adenosine and iloprost. Previous studies have suggested an inhibitory action of cAMP on PGI<sub>2</sub> release. However, this conclusion was based solely on the inhibitory effect of IBMX [23]. It is now strengthened by our results with the receptor-mediated cAMP-raising agents iloprost and adenosine. Our observations imply that PGI<sub>2</sub> can inhibit its own release from endothelial cells, in an autocrine manner. The inhibitory effect of IBMX on PGI<sub>2</sub> release is greater than that of other cAMP-raising agents. For instance, the effect of IBMX on PGI<sub>2</sub> release was greater than that of forskolin, while the effect of these two agents on cAMP cellular content is a 2-fold and 4-fold increase respectively [7]. A possible explanation is that IBMX raises not only cAMP but also cGMP levels (by inhibition of cGMP degradation) [24], which could be involved in the regulation of PGI<sub>2</sub> release. We did not pursue this hypothesis given the limitations of our culture system for the investigation of cGMP-mediated signaling (see below).

The possible effects of nitric oxide/cGMP-dependent signaling on vWf release are an unsettled issue. Using a rat hindlimb perfusion system, Tranquille and Emeis reported an inhibitory effect of sodium nitroprusside and atrial natriuretic peptide – but not of 8-bromo-cGMP – on vWf and t-PA release [18]. In cat coronary artery segments, thrombin-induced endothelial exocytosis could be inhibited by endothelin-1 in an NO-dependent manner [25]. In contrast, exercise-induced increases in plasma vWf levels in healthy humans appear to be mediated in part by NO, since they are inhibited by the NO synthase inhibitor L-NMMA [26]. We failed to observe an effect of either NO donors or 8-bromo-cGMP on vWf release in cultured HUVECs. However, this negative result does not exclude a role for NO-mediated signaling in vWf release. Indeed, in contrast to aortic endothelial cells, HUVECs do not express cGMP-dependent protein kinase type I, the activation of which is thought to inhibit thrombin-induced calcium mobilization [27]. These cells thus appear to be insensitive to an inhibitory effect of NO-dependent signaling on vWf release.

In summary, our results demonstrate that the prostacyclin analog iloprost, like other cAMP-raising agents, induces vWf release from cultured HUVECs, and potentiates thrombin-induced vWf release. This is a selective effect since cAMP-raising agents – including iloprost – inhibit PGI<sub>2</sub> release. The latter finding suggests that PGI<sub>2</sub> can inhibit its own release in an autocrine manner and provides a mechanism for the differential regulation of two endothelial calcium-dependent effector systems.

**Acknowledgements:** The technical assistance of Nicole Aebischer and Marcela Klein is gratefully acknowledged. This work was supported by Grants 32-41941.94 (U.M.V.) and 31-42-295.94 (U.L.) from the Swiss National Science Foundation. U.M.V. is the recipient of a SCORE subsidy from the Swiss National Science Foundation.

## References

- [1] Wagner, D.D. (1990) *Annu. Rev. Cell Biol.* 6, 217–246.
- [2] Birch, K., Ewenstein, B.M., Golan, D.E. and Pober, J.S. (1994) *J. Cell Physiol.* 160, 545–554.
- [3] Birch, K.A., Pober, J.S., Zavoico, G.B., Means, A.R. and Ewenstein, B.M. (1992) *J. Cell Biol.* 118, 1501–1510.
- [4] Frearson, J.A., Harrison, P., Scrutton, M.C. and Pearson, J.D. (1995) *Biochem. J.* 309, 473–479.
- [5] Wheeler-Jones, C.P.D., May, M.J., Morgan, A.J. and Pearson, J.D. (1996) *Biochem. J.* 315, 407–416.
- [6] Michel, T. and Feron, O. (1997) *J. Clin. Invest.* 100, 2146–2152.
- [7] Vischer, U.M. and Wollheim, C.B. (1997) *Thromb. Haemost.* 77, 1182–1188.
- [8] Vischer, U.M. and Wollheim, C.B. (1998) *Blood* 91, 118–127.
- [9] Namba, T., Oida, H., Sugimoto, Y., Kakizuka, A., Nedishi, M., Ichikawa, A. and Narumiya, S. (1994) *J. Biol. Chem.* 269, 9986–9992.
- [10] Langelier, E.G. and van Hinsbergh, V.W.M. (1991) *Am. J. Physiol.* 260, C1052–C1059.
- [11] Jornot, L. and Junod, A. (1993) *Am. J. Physiol.* 264, L482–L489.
- [12] Vischer, U.M., Jornot, L., Wollheim, C.B. and Theler, J.M. (1995) *Blood* 85, 3164–3172.
- [13] Lang, U. and Vallotton, M.B. (1989) *Biochem. J.* 259, 477–484.
- [14] Rickles, F.R., Hoyer, L.W., Rick, M.E. and Ahr, D.J. (1976) *J. Clin. Invest.* 57, 1618–1625.
- [15] Cohen, R.J., Epstein, S.E., Cohen, L.S. and Dennis, L.H. (1968) *Lancet* 2, 1264–1266.
- [16] Åmmälä, C., Ashcroft, F.M. and Rorsman, P. (1993) *Nature* 363, 356–358.
- [17] Vajanaphanich, M., Schultz, C., Tsien, R.Y., Traynor-Kaplan, A.E., Pandol, S.J. and Barrett, K.E. (1995) *J. Clin. Invest.* 96, 386–393.
- [18] Tranquille, N. and Emeis, J.J. (1993) *Thromb. Haemost.* 69, 259–261.
- [19] Wigley, F.M., Wise, R.A., Seibold, J.R., McCloskey, D.A., Kujala, G., Medsger, T.A., Steen, V.D., Varga, J., Jimenez, S. and Mayes, M. (1994) *Ann. Intern. Med.* 120, 199–206.
- [20] Friedman, R., Mears, G. and Barst, R.J. (1997) *Circulation* 96, 2782–2784.
- [21] Boyer-Neumann, C., Brenot, F., Wolf, M., Peynaud-Debayle, E., Duroux, P., Meyer, D., Anflès-Cano, E. and Simonneau, G. (1995) *Thromb. Haemost.* 73, 727–728.
- [22] Galbusera, M., Zoja, C., Donadelli, R., Paris, S., Morigi, M., Benigni, A., Figliuzzi, M., Remuzzi, G. and Remuzzi, A. (1997) *Blood* 90, 1558–1564.
- [23] Adams-Brotherton, A.F. and Hoak, J.C. (1982) *Proc. Natl. Acad. Sci. USA* 79, 495–499.
- [24] Beavo, J.A. (1995) *Physiol. Rev.* 75, 725–748.
- [25] Murohara, T. and Lefer, A.M. (1996) *Blood* 88, 3894–3900.
- [26] Jilma, B., Dirnberger, E., Eichler, H.G., Matulla, B., Schmetterer, L., Kapiotis, S., Speiser, W. and Wagner, O.F. (1997) *Thromb. Haemost.* 78, 1268–1271.
- [27] van Hinsbergh, V.W.M. (1997) *Arterioscl. Thromb. Vasc. Biol.* 17, 1018–1023.