Regulated von Willebrand Factor Secretion Is Associated With Agonist-Specific Patterns of Cytoskeletal Remodeling in Cultured Endothelial Cells

Ulrich M. Vischer, Holger Barth, Claes B. Wollheim

Abstract—von Willebrand factor (vWF), an adhesive glycoprotein involved in primary hemostasis, is stored and released from endothelial secretory granules called Weibel-Palade bodies. Regulated secretion occurs in reaction either to [Ca\(^{2+}\)];-raising agents (histamine or thrombin) or to cAMP-raising agents (epinephrine, adenosine, or forskolin). We investigated the pattern of release and the cytoskeletal requirements for secretion in response to these 2 classes of agonists. Secretion induced by [Ca\(^{2+}\)];-raising agents involves peripheral and central granules and is inhibited by colchicine-induced microtubule disruption. It is accompanied by Rho-dependent stress fiber formation and cell retraction. Secretion and remodeling occur in the same individual cells. However, secretion is potentiated by cytochalasin E and C3 toxin, indicating that stress fiber formation antagonizes vWF secretion. In contrast, vWF secretion induced by cAMP-raising agents involves the release of only peripheral granules (implying less vWF release on a per cell basis) and is not inhibited by microtubule disruption. cAMP-mediated secretion is accompanied by disruption of stress fibers, strengthening of the cortical actin rim, and preservation of cell-cell contacts. It is unaffected by cytochalasins or C3 toxin. In contrast to [Ca\(^{2+}\)];-raising agents, cAMP-raising agents induce secretion without cell retraction/intercellular gap formation. Thus, they are likely to play a physiological role in the regulation of endothelial vWF secretion and, therefore, of plasma vWF levels. (Arterioscler Thromb Vasc Biol. 2000;20:883-891.)

Key Words: von Willebrand factor | endothelial cells | cytoskeleton

on Willebrand factor (vWF) is an adhesive glycoprotein involved in primary hemostasis, ie, the adhesion of platelets to the vascular subendothelium. vWF deficiency leads to von Willebrand’s disease, a common bleeding disorder. vWF is synthesized in vascular endothelial cells (ECs) and megakaryocytes, stored in endothelial and platelet secretory granules, and released into the circulation in a regulated manner. Most circulating vWF is derived from the endothelium. However, the regulation of plasma vWF levels, ie, of endothelial secretion, is still incompletely understood. In ECs, vWF is stored in specialized secretory granules called Weibel-Palade (WP) bodies.1 Exocytosis from these granules occurs in response to several agonists. One class of agonists, identified in vitro studies, includes thrombin, histamine, and other mediators of inflammation and/or thrombosis.2-4 Their effect seems to be mediated by a rise in cytosolic free Ca\(^{2+}\) ([Ca\(^{2+}\)];).2,5 A rise in [Ca\(^{2+}\)]; induced by Ca\(^{2+}\); ionophores, such as A23187 or ionomycin, is sufficient to induce acute vWF release.6,7 Thrombin-induced vWF release is associated with a rise in [Ca\(^{2+}\)];, and can be prevented by preincubation with intracellular Ca\(^{2+}\); chelators. A role for calmodulin in mediating the Ca\(^{2+}\); effect is suggested by the inhibitory action of a calmodulin-binding peptide on vWF release in permeabilized ECs.2 The molecular mechanisms distal to the activation of calmodulin have so far not been studied.

A second mechanism for regulated vWF secretion was suggested by early observations that epinephrine infusion and stress situations (such as physical activity, hypoglycemia, and central nervous system stimulation) result in a rapid short-lived increase in plasma vWF levels.8,9 None of the known [Ca\(^{2+}\)];-raising agonists are likely to mediate these effects. However, several cAMP-raising agents have recently been shown to induce vWF release from cultured ECs.10,11 These include forskolin (an activator of adenylate cyclase), cell-permeant cAMP analogues (eg, 8-bromo-cAMP), epinephrine (acting via β-adrenergic receptors coupled to adenylate cyclase), prostacyclin, and adenosine. A rise in cellular cAMP is sufficient to induce vWF release, independent of a rise in [Ca\(^{2+}\)];. We have proposed that cAMP-mediated secretion, in particular that due to adrenergic activation, is an important mechanism in the physiological regulation of plasma vWF levels (reviewed in Reference 12).

ECs have multiple effector systems that are involved not only in hemostasis but also in the regulation of vascular tone, leukocyte traffic, and vascular permeability.13 The differen-
tial regulation of these various systems is an important theme in EC physiology. EC contractile state is a key determinant of vascular permeability to ions and macromolecules. Exposure of human umbilical vein ECs (HUVECs) to thrombin and histamine results in rapid contraction, which is secondary to the rearrangement of actin and myosin into stress fibers. Like secretion, the contractile response is dependent on a rise in [Ca\(^{2+}\)]\(_i\) and calmodulin. Ca\(^{2+}\)/calmodulin activates myosin light chain kinase, a key enzyme in the contractile process. Thrombin-induced cytoskeletal remodeling and vWF secretion appear to have a similar rapid time course. Because secretion and cytoskeletal remodeling are regulated by a rise in [Ca\(^{2+}\)], there could be a mechanistic link between these 2 events, as has been suggested in several other cell types. In contrast, cAMP-raising agents induce vWF secretion, yet they induce a very different pattern of cytoskeletal remodeling (eg, disruption of stress fibers) that is possibly related to the inhibition of myosin light chain kinase. The changes are associated with a decrease in vascular permeability.

The purpose of the present study was to clarify the effects of various vWF secretagogues on cytoskeletal remodeling and to investigate whether there is any mechanistic link between secretion and contraction in ECs.

**Methods**

**Materials**

RPMI 1640 was from GIBCO-BRL, and FCS and collagenase were from Seromed. EC growth supplement was from Upstate Biotechnology Inc. Thrombin, histamine, A23187, forskolin, and isobutylmethylxanthine (IBMX) were from Sigma Chemical Co. Anti-tubulin DM-1A monoclonal antibodies were also from Sigma. Rhodamine-phalloidin was from Fluka. Anti-vWF antibodies were from Dako, and anti-myosin monoclonal antibodies 2F12.A9 were from Immunotech.
Cell Culture

Primary cultures of HUVECs were obtained from individual human umbilical veins by collagenase digestion as previously described. They were grown in medium RPMI 1640 supplemented with 10% FCS, 90 μg/mL heparin, and 15 μg/mL EC growth supplement. Cells were used during passages 1 or 2. Tissue culture dishes and the 24-well plates (Costar) were coated with 0.1% gelatin. To improve cell adhesion to glass coverslips, these were coated with glutaraldehyde–cross-linked gelatin as described.19

Secretion Studies

Confluent monolayers of HUVECs grown in 24-well dishes were washed 3 times and preincubated in 0.5 mL Krebs-Ringer-bicarbonate buffer (mmol/L: NaCl 120, KCl 4.75, KH₂PO₄ 1.2, MgSO₄ 0.6, CaCl₂ 1.2, NaHCO₃ 25, and HEPES 25, pH 7.4 [KRBBH], supplemented with 0.1% BSA) for 5 minutes at 37°C. After a fourth wash, cells were incubated in 0.3 to 0.5 mL KRBBH with the different agents. All pharmacological agents were dissolved directly in incubation medium or in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in the incubation medium did not exceed 0.2%, a concentration that has no effect on vWF release (not shown). The incubation medium was cleared of cell debris by centrifugation, and the individual supernatants were stored at −20°C until the time of assay.

vWF Measurements

vWF was measured by ELISA as described previously. A standard curve was constructed from serial dilutions of normal pooled plasma; a plasma concentration of 10 μg/mL was assumed. Results are usually expressed in nanograms per well per time unit. We observed considerable variations in cellular vWF content and rate of secretion between cell batches. Therefore, when necessary, the results are expressed in relative values, ie, as a percentage of release from unstimulated control cells from the same cell preparation. Unless indicated otherwise, results are shown as mean ± SEM. Statistical analysis was performed by use of the 2-tailed paired Student t test.

Immunofluorescence

HUVECs grown on glass coverslips were fixed in 3.7% formaldehyde, followed by permeabilization in 0.5% Triton X-100. For myosin staining, the cells were fixed in 100% methanol for 4 minutes at −20°C. Antibodies were diluted in PBS containing 2% BSA. The following antibodies were used: rabbit anti-vWF at 1:3000, monoclonal anti-tubulin at 1:200, and monoclonal anti-myosin 2F12.A9 at 1:20. This antibody was raised against quail oviduct but recognizes platelet myosin. By Western blot, it recognizes a 205-kDa band in HUVECs. This band comigrates with nonmuscle myosin IIA, detected with a specific affinity-purified rabbit antibody (kindly provided by Prof G. Gabbiani, Figure 2. Correlation between agonist-induced vWF release and cytoskeletal remodeling. Confluent HUVECs were exposed to control buffer for 20 minutes (a and a*), 10⁻⁵ mol/L histamine for 5 minutes (b and b*), or 10 μmol/L forskolin/100 μmol/L IBMX for 20 minutes (c and c*). The cells were stained by sequential incubations with anti-myosin monoclonal antibodies, fluorescein-conjugated anti-mouse IgGs, rabbit anti-vWF antibodies, and rhodamine-conjugated anti-rabbit IgGs. Identical fields are shown in panels a and a*, in panels b and b*, and in panels c and c*. Left panels show the vWF (rhodamine) stain; the right panels show the myosin (fluorescein) stain. In single-stain controls, the rhodamine signal was not visible in the fluorescein channel and vice versa (not shown). In unstimulated cells, WP bodies were visualized as rod-shaped granules reacting with anti-vWF antibodies (a), and myosin was stained in a diffuse cytosolic pattern (a*). After histamine stimulation, many cells were virtually depleted of WP bodies (b) and displayed rearrangement of myosin along stress fibers (b*). There was a one-to-one correspondence between granular depletion and myosin rearrangement in individual cells. After thrombin stimulation, a greater proportion of cells had undergone myosin rearrangement, making the granular depletion/myosin rearrangement correspondence more difficult to establish (not shown). A similar pattern was seen after 20 minutes. After forskolin/IBMX stimulation, only peripheral WP bodies had disappeared, and complete granule depletion was very rare (c); the myosin distribution was not affected (c*). Original magnification ×400.
Results

**Differential Effects of [Ca\textsuperscript{2+}]- and cAMP-Raising Agents on Actin and Myosin Distribution**

The effects of the secretion agonists on actin and myosin rearrangement in confluent monolayers of HUVECs were compared. After exposure to the agonist, the cells were fixed, permeabilized, and stained for F-actin with rhodamine-phalloidin or for myosin II with a monoclonal anti-myosin antibody (see Methods) (Figure 1). Resting cells exhibited a thin cortical F-actin rim at their margins. This rim was thin enough to make identification of cell-cell junctions uncertain at times. Most cells contained stress fibers, although these were inconspicuous and in apparently random orientation. Myosin II was diffusely distributed throughout the cytoplasm, without obvious localization. After a 20-minute exposure to either histamine or thrombin, actin had reorganized into prominent stress fibers, typically arranged in a parallel pattern along the longitudinal axis of the cell. Cells had retracted, leaving intercellular gaps. These gaps were much more prominent after thrombin than after histamine stimulation. Myosin II was redistributed to the underlying stress fibers in a bandlike pattern. Qualitatively similar changes in actin and myosin distribution were observed after only 5 minutes (eg, see Figure 2b). A very different pattern was seen after exposure to forskolin/IBMX. There was a rapid disappearance of most stress fibers, and the peripheral actin rim became thicker and more linear, providing a clear outline of cell-cell junctions. The absence of intercellular gaps was striking. Forskolin-induced actin redistribution was clearly visible at 5 and 20 minutes (not shown). There were no consistent changes in myosin II distribution, which remained diffuse.

**Different Patterns of Secretory Granule Release in Response to [Ca\textsuperscript{2+}]- and cAMP-Raising Agents**

Because [Ca\textsuperscript{2+}]- and cAMP-raising agents induce distinct patterns of cytoskeletal remodeling, we next investigated whether they also induce distinct patterns of granule release. Confluent HUVECs treated with histamine, thrombin, or forskolin/IBMX were fixed and double-stained for vWF and myosin (Figure 2). vWF staining revealed WP bodies as typical rod-shaped immunoreactive granules. In control cells, these granules were diffusely spread throughout the cytoplasm (Figure 2a), in agreement with our previous report.\textsuperscript{4} After exposure to histamine, a marked granule depletion occurred in responsive cells. Many cells were entirely depleted of their granules. Strikingly, all depleted cells had undergone cytoskeletal rearrangement, as evident from the myosin redistribution to stress fibers. The granule content was unaffected in cells in which myosin had remained in a diffuse cytosolic pattern (Figure 2b and 2b′). The pattern with forskolin was clearly different. Forskolin caused the release of only peripheral granules. Complete cell emptying was rare; ie, the number of cells entirely devoid of granules was not different in forskolin-treated than in control cells. Again, forskolin-induced secretion was not accompanied by myosin redistribution (Figure 2c and 2c′). These observations suggest that [Ca\textsuperscript{2+}]- and cAMP-raising agents can induce granule-membrane fusion but that only [Ca\textsuperscript{2+}]-raising agents induce granule transport from the trans-Golgi network area to the cell membrane. The one-to-one correspondence of histamine-induced vWF secretion and myosin redistribution in individual cells raises the possibility of a mechanistic link between secretion and cytoskeletal remodeling.

**Effects of Cytochalasins on vWF Secretion**

Cytochalasins prevent actin polymerization and are thus predicted to disrupt the cortical actin rim and to prevent the formation of stress fibers. Therefore, we tested the effects of cytochalasins on agonist-induced vWF release from confluent HUVECs. In preliminary experiments, we observed that cytochalasin B (0.5 to 10 µg/mL) and cytochalasin E (CCE, 0.1 to 2.0 µg/mL) potentiated thrombin-induced vWF release. We next measured vWF release after a 30-minute preincubation with 0.1 to 0.15 µg/mL CCE, followed by a 20-minute incubation with histamine, thrombin, A23187, forskolin/IBMX, and adenosine/IBMX (Figure 3). CCE caused a small
increase in vWF release from unstimulated cells. Surprisingly, preincubation with CCE potentiated the secretory response to thrombin but not to any of the other tested agents. The effect of CCE on F-actin distribution was determined by staining with rhodamine-phalloidin (Figure 4). After preincubation with CCE (Figure 4a and 4a’), the peripheral actin rim was lost or became discontinuous, and stress fibers disappeared. CCE prevented the formation of stress fibers in response to thrombin (Figure 4b and 4b’) and histamine (Figure 4c and 4c’). As in unstimulated cells, CCE caused a loss of continuity of the peripheral actin rim after forskolin stimulation (Figure 4d and 4d’). The easiest interpretation of these findings is that stress fiber formation and/or the accompanying cell retraction actually impair secretion in response to \([\text{Ca}^{2+}]_{i}\)-raising agents. CCE pretreatment prevents stress fiber formation, but its potential effect on secretion is counteracted by other inhibitory effects. The effect on secretion is seen only with thrombin, which is the most effective agent in terms of stress fiber formation or cell retraction (see Figure 1). At late time points (ie, >5 minutes), vWF secretion is stronger in response to histamine than to thrombin (not shown), a finding that is compatible with a pronounced inhibitory effect of remodeling on thrombin-induced vWF secretion.

**Figure 4.** Effect of CCE on F-actin distribution. Confluent HUVECs were exposed to control buffer \((a, b, c,\) and \(d) or to 0.1 to 0.15 \(\mu \text{g/mL}\) CCE \((a’, b’, c’,\) and \(d’)) for 30 minutes at 37°C before stimulation for 20 minutes with buffer alone \((a \text{ and } a’), 0.5 \text{ U/mL thrombin } (b \text{ and } b’), 10^{-5} \text{ mol/L histamine } (c \text{ and } c’), \text{ or } 10 \mu \text{mol/L forskolin/100 } \mu \text{mol/L IBMX } (d \text{ and } d’).\) CCE caused the partial disruption of cell-cell contacts and rearrangement of F-actin into amorphous clusters in controls and forskolin/IBMX-treated cells; the peripheral actin rim became discontinuous \((a’ \text{ and } d’).\) CCE also prevented the formation of stress fibers in response to thrombin \((b’ \text{ and } c’)\) and histamine \((c’).\) Original magnification \(x400.\)

**Involvement of Rho in vWF Secretion: Effect of C3-Like Toxin**

Our experiments with CCE suggested that stress fiber formation/cell retraction impairs vWF release in response to \([\text{Ca}^{2+}]_{i}\)-raising agents. However, CCE not only prevents stress fiber formation but also disrupts other F-actin structures. Therefore, we looked for a more specific inhibitor of stress fiber formation.

Stress fiber formation is dependent on the small GTPase Rho. The C3 exotoxin, an inhibitor of Rho, would therefore be predicted to prevent stress fiber formation without disrupting other F-actin structures. Because C3 is poorly cell permeant, we used the recently described C3-C2IN chimeric toxin, which enters the cells by using the C2 uptake system (see Methods). The effect of this toxin on agonist-induced actin redistribution was examined (Figure 5). C3-C2IN (100 ng/mL) and C2IIa (100 ng/mL) were directly added to complete culture medium for 2, 4, and 6 hours before stimulation with secretion agonists. The cells were then stained with rhodamine-phalloidin. At all 3 time points, the chimeric toxin caused the disappearance of stress fibers and a strengthening of the peripheral actin rim (Figure 5c). The pattern observed was highly reminiscent of that seen after treatment with forskolin/IBMX (compare Figure 5c with...
The chimeric toxin also prevented the formation of stress fibers in response to histamine (Figure 5d); the persistence of the peripheral actin rim and the absence of intercellular gaps even after histamine treatment were quite remarkable. Only a small number of cells (≈20%) displayed histamine-induced stress fiber formation, suggesting that this subpopulation of cells did not take up the toxin. Similar effects of the chimeric toxin were observed on thrombin- and A23187-induced stress fiber formation (not shown). Thus, the C3-like toxin prevented stress fiber formation without causing a disruption of the peripheral actin rim or nonspecific cell deformation.

Exposure of HUVECs to A23187 induced the formation of stress fibers, but these were less abundant than after histamine or thrombin and were frequently oriented in a circumferential rather than a parallel manner (Figure 5e and 5f). It is likely that in addition to raising \([Ca^{2+}]_i\), thrombin and histamine acutely activate Rho. However, we did not determine whether histamine, thrombin, or A23187 caused GTP incorporation into Rho, in view of the fact that metabolic labeling of HUVECs would have required prohibitive quantities of cells.

We next tested the effect of a 4-hour pretreatment with the C3-C2IN toxin on agonist-induced vWF release in confluent HUVECs (Figure 6). This pretreatment did not cause cellular vWF depletion, as indicated by measurements of vWF in cell lysates (not shown). The C3-C2IN toxin caused a marked potentiation of the secretory responses to thrombin, histamine, and A23187. In contrast, the responses to forskolin/IBMX and adenosine/IBMX were not affected by the pretreatment.

### Effect of Microtubule Disruption on vWF Release

An earlier report has shown that disruption of microtubules with colchicine inhibits vWF secretion induced by A23187 and thrombin.\(^{21}\) We extended these studies to compare the effect of colchicine on vWF secretion in response to \([Ca^{2+}]_i\)- and cAMP-raising agents (Figure 7). Pretreatment with colchicine (1 \(\mu\)mol/l) for 30 to 60 minutes caused a significant (>60%) inhibition of both histamine- and thrombin-induced vWF release (\(P<0.005\), \(n=4\) and 8 respectively). A23187-induced vWF secretion was inhibited by 35%; this effect was consistently observed, although it failed to reach statistical significance (\(P=0.11\), \(n=4\)). In contrast, forskolin-induced vWF was not affected by colchicine pretreatment (−11%, \(P=0.65\), \(n=6\)). We verified the effect of colchicine on microtubules by indirect immunofluorescence with an antibody to tubulin (Figure 8). Microtubules were visualized as typical radially oriented fibers. Microtubule morphology was not affected by treatment with any of the secretion agonists. Colchicine pretreatment completely abolished the microtubule pattern. Thus, microtubule disruption inhibited the secretory response to thrombin and histamine but not to forskolin.

### Discussion

Previous work has suggested the existence of 2 classes of vWF-releasing agonists. The first, exemplified by thrombin
eral granules, whereas histamine caused complete granule release in a significant proportion of responsive cells. These findings strongly suggest that both [Ca\(^{2+}\)]- and cAMP-raising agents can induce granule fusion with the cell membrane, whereas only [Ca\(^{2+}\)]-raising agents can recruit granules from the Golgi area to the cell membrane.

We also observed quite distinct remodeling patterns in response to [Ca\(^{2+}\)]- and cAMP-raising agents. Exposure to thrombin and histamine resulted in the rapid redistribution of actin and myosin into prominent stress fibers and cell retraction. In contrast, forskolin caused the disruption of existing stress fibers and a strengthening of the cortical actin rim, accompanied by a tightening of cell-cell contacts.

Thrombin and histamine induced rapid stress fiber formation, in agreement with earlier reports.\(^{15,16}\) Raising [Ca\(^{2+}\)], with A23187 induced less abundant stress fibers, suggesting that additional thrombin- or histamine-induced signaling events (other than a rise in [Ca\(^{2+}\)]) are involved. Stress fiber formation in response to thrombin and histamine was abolished by pretreatment with the C3-C2IN toxin. This finding strongly suggests that Rho is involved in this process. Rho-GTP activates a specific kinase, p160ROCK.\(^{22}\) Two of the substrates for this kinase are myosin light chain and myosin light chain phosphatase, which are known to regulate the assembly of actin filaments.\(^{23,24}\) It is very likely that thrombin and other [Ca\(^{2+}\)]-raising agents acutely activate Rho, but this conclusion awaits the direct demonstration (and quantification) of agonist-induced GTP incorporation into the Rho protein. At odds with our results, Vouret-Craviari et al\(^{25}\) recently reported that C3 pretreatment induced the formation of intercellular gaps and did not abolish thrombin-induced cell retraction. Our results may be dependent on the use of the C3-C2IN chimeric toxin, which allows more rapid cell loading (2 to 6 hours), thus avoiding possible long-term adaptive mechanisms.

We found a definite correlation between stress fiber formation and secretion in individual cells after histamine and thrombin stimulation. Furthermore, the time courses for histamine-induced vWF secretion and stress fiber formation were quite similar. These observations suggest a mechanistic link between these 2 events, as has been suggested in other cell types.\(^{26,27}\) However, preventing stress fiber formation by CCE pretreatment potentiated thrombin-induced vWF release. Pretreatment with the more specific C3-C2IN toxin prevented stress fiber formation and potentiated vWF release in response to thrombin, histamine, and A23187 but not to cAMP-raising agents. These findings strongly suggest that stress fibers actually antagonize vWF secretion. The likeliest explanation is that stress fibers are a hindrance to granular movement from the Golgi area to the cell membrane. An alternative explanation is that cell retraction (rather than stress fiber formation, per se) prevents secretion. This could result from cell rounding and the consequent impairment of the contact between peripheral granules and the cell membrane. This explanation is unlikely in the case of histamine, which causes little retraction. However, it may account for the weaker secretory response to thrombin than to histamine, as shown at the later points in the time-course study.

Forskolin-induced cytoskeletal remodeling consists of the disruption of existing stress fibers and a strengthening of the cortical actin rim, accompanied by a tightening of cell-cell contacts. These morphological changes were reminiscent of...
those induced by the C3-C2IN toxin. It is worth noting that cAMP is inhibitory to both myosin light chain kinase and Rho, which are closely involved in stress fiber formation. However, the C3-C2IN toxin failed to mimic forskolin-induced secretion. Furthermore, we observed dissociated time courses, with the actin remodeling clearly preceding secretion. These observations suggest that Rho inhibition and stress fiber disruption are not sufficient for cAMP-mediated vWF secretion.

It was previously shown that colchicine inhibits thrombin-induced vWF release, suggesting that microtubules are required for secretion. In isolated islet cells, colchicine blocked the delayed but not the immediate glucose-induced insulin release, implying that microtubules are required for granule migration to the cell surface. We again observed that colchicine causes a significant inhibition of thrombin- and histamine-induced vWF release. However, forskolin-induced secretion was not affected. These observations are in keeping with the notion that [Ca^{2+}]-raising agents recruit centrally located WP bodies, an effect dependent on microtubules for granular migration. In contrast, forskolin-induced vWF release is resistant to microtubular disruption, consistent with the immunofluorescence data showing that forskolin induces the release of peripheral granules only.

In summary, the present study further distinguishes 2 classes of secretion agonists. Secretion induced by [Ca^{2+}]-raising agents (thrombin and histamine) involves peripheral and central granules and is accompanied by massive stress fiber formation and cell retraction. Stress fiber formation appears to antagonize vWF secretion. In contrast, secretion induced by cAMP-raising agents involves the release of only peripheral secretory granules and is unaffected by microtubular disruption. cAMP-mediated secretion is accompanied by disruption of stress fibers, strengthening of the cortical actin rim, and tightening of cell-cell contacts. None of these changes appear to be essential for secretion. In vivo, most [Ca^{2+}]-raising agents are mediators of inflammation or thrombosis, usually acting in a regional manner. In this setting, vWF secretion is expected to be accompanied by cell retraction, possibly resulting in edema formation or exposure of the subendothelium (and consequent thrombotic events). In contrast, cAMP-raising agents are expected to induce a smaller vWF secretory response on a per cell basis and to preserve endothelial cell-cell junctions. These are the features...
expected from cAMP-raising agents such as epinephrine, which act in a systemic rather than a regional manner. Thus, our results reinforce our hypothesis that endothelial cAMP-mediated vWF secretion is an important mechanism in the physiological regulation of plasma vWF levels.

Acknowledgments

This study was supported by grant No. 3200 052667.97 and a SCORE subsidy (to U.M.V.) from the Swiss National Science Foundation. We thank Nicole Aebischer for skilled technical assistance.

References

Regulated von Willebrand Factor Secretion Is Associated With Agonist-Specific Patterns of Cytoskeletal Remodeling in Cultured Endothelial Cells
Ulrich M. Vischer, Holger Barth and Claes B. Wollheim

doi: 10.1161/01.ATV.20.3.883
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/20/3/883

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/