Differing Polarity of the Constitutive and Regulated Secretory Pathways for von Willebrand Factor in Endothelial Cells

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Abstract. von Willebrand factor (vWf) is secreted from endothelial cells by one of two pathways—a constitutive pathway and a regulated pathway originating from the Weibel–Palade bodies. The molecular form of vWf from each of these pathways differs, with the most biologically potent molecules being released from Weibel–Palade bodies (Loesberg, C., M. D. Gonsalves, J. Zandbergen, C. Willems, W. G. Van Aken, H. V. Stel, J. A. Van Mourik, and P. G. DeGroot. 1983. *Biochim. Biophys. Acta.* 763:160–168; Sporn, L. A., V. J. Marder, and D. D. Wagner. 1987. *Cell.* 46:185–190). We investigated the polarity of the two secretory pathways using human umbilical vein endothelial cells cultured on polycarbonate membrane filters which allowed sampling of media from both the apical and basolateral compartments. After metabolic labeling of cells, vWf (constitutively secreted during a 10-min period or released during a 10-min treatment with a secretagogue) was purified from the apical and basolateral chambers and subjected to gel analysis. Approximately equal amounts of vWf were constitutively secreted into both chambers, and therefore this secretory pathway appeared to be nonpolarized. On the contrary, an average of 90% of vWf released from Weibel–Palade bodies after treatment with the calcium ionophore A23187 or PMA appeared in the basolateral chamber, indicating that the regulated pathway of secretion is highly polarized. Thrombin, a secretagogue which promotes disruption of the endothelial monolayer, led to release of vWf from cells with no apparent polarity. The presence of microtubule-depolymerizing agents nocodazol and colchicine inhibited the polarized release of vWf. Ammonium chloride treatment did not disrupt the polarity of the regulated secretory pathway, indicating that maintenance of low pH in intracellular compartments was not required for the polarized delivery of preformed Weibel–Palade bodies to the plasma membrane.

THE polarity of the epithelial cell has been a subject of many recent investigations, with regard to both the specific functions localized to the apical and basolateral cell surfaces as well as the cellular machinery involved in directing and maintaining the polarized phenotype (Matlin, 1986; Simons and Fuller, 1985). Weak bases, such as ammonium chloride, have been shown to interfere with polarized secretion of proteins and proteoglycans from epithelial cells (Caplan et al., 1987). This indicates either that the sorting process requires an acidic intracellular compartment or that the weak base interferes with vesicular traffic or correct membrane fusion. Vascular endothelial cells, exposed on the luminal surface to the bloodstream and on the abluminal surface to the basement membrane, are polarized cells with respect to their plasma membrane constituents (Simionescu et al., 1981; Nakache et al., 1985; Horvat et al., 1986; Muller and Gimbrone, 1986), protein secretion (Zerwes and Risau, 1987), and uptake of molecules (Palade, 1960). The present study investigates the polarity of the regulated vs. the constitutive secretory pathway for von Willebrand factor (vWf)[1] in the endothelial cell and the effect of a weak base on the polarized release of presorted vWf molecules.

vWf, synthesized by endothelial cells (Jaffe et al., 1973) and megakaryocytes (Nachman et al., 1977; Sporn et al., 1985), is a large adhesive glycoprotein that functions in the hemostatic process (Tschopp et al., 1974; Sakariassen et al., 1979; Hovig and Stormorken, 1974; Turitto et al., 1984). vWf undergoes extensive posttranslational modification which includes dimerization and initial glycosylation in the endoplasmic reticulum, carbohydrate processing, sulfation, multimerization of dimers, and prosequence cleavage in Golgi and post-Golgi compartments (Handin and Wagner, 1988). The largest disulfide-bonded multimers of vWf produced by endothelial cells have molecular weights estimated to be 10–20 million (Zimmerman et al., 1983) and bind the most avidly to the platelets (Zimmerman et al., 1983) as well

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1. *Abbreviation used in this paper:* vWf, von Willebrand factor.

as to the extracellular matrix (Sporn et al., 1987). All multimeric sizes, however, can serve as carriers for circulating factor VIII, the antihemophilic factor (Moake et al., 1983).

vWf is secreted from endothelial cells via one of two pathways (Loesberg et al., 1983). The regulated pathway of secretion originates from the Weibel–Palade bodies, the endothelial cell–specific storage vesicles for vWf (Wagner et al., 1982). Release of vWf from this intracellular pool occurs rapidly after stimulation of the cells with such agents as thrombin (Levine et al., 1982; Loesberg et al., 1983), the calcium ionophore A23187 (Loesberg et al., 1983), PMA (Loesberg et al., 1983), histamine (Hamilton and Sims, 1987), and fibrin (Ribes et al., 1987). It is likely that vWf secreted by the constitutive secretory pathway leaves the cell by a bulk flow process along with other endothelial cell secretory proteins. Pulse–chase studies have shown that an average of 5% of newly synthesized vWf is sorted into the Weibel–Palade bodies, while the remainder is constitutively secreted (Sporn et al., 1986).

The molecular forms of vWf originating from these two secretory pathways differ. The regulated secretory pathway secretes the largest multimeric forms of the protein, composed of processed subunits; the predominantly small multimeric forms, containing some pro-vWf subunits, are secreted constitutively (Sporn et al., 1986; Ewenstein et al., 1987). The intracellular movement of Weibel–Palade bodies during the process of regulated secretion appears to involve the microtubular cytoskeleton, since regulated secretion is inhibited by microtubule-depolymerizing agents. Constitutive secretion, on the other hand, is not significantly affected by these agents (Sinha and Wagner, 1987). We report that in addition to the above described differences between the pathways, polarity with respect to the apical and basolateral plasma membrane is also different.

Materials and Methods

Cells and Culture Conditions

Endothelial cells were harvested from human umbilical vein by mild proteolytic digestion as described (Wagner et al., 1982; Gimbrone et al., 1974). Freshly isolated cells were plated on Transwell™ cell culture chamber inserts (Costar, Cambridge, MA) which were gelatin coated by incubating for 1 h at 37°C with a solution of 1.5% porcine gelatin (Sigma Chemical Co., St. Louis, MO) in PBS and rinsed with culture medium. The Transwell™ inserts are composed of a tissue culture-treated polycarbonate membrane 10 µM thick, 24.5 mm in diameter, with a pore size of 3.0 µM, imbedded in a solid plastic support. When placed in a 6-well cluster plate (Costar) sampling from both the apical and basolateral chambers is allowed. For metabolic labeling, 4–7-d-old cells were placed in 25 µCi/ml [³⁵S]cysteine (600 Ci/mmol; Amersham Corp., Arlington Heights, IL) for 3 d; for studies of constitutive secretion, the cells were placed in 100 µCi/ml for 2 h. Culture media volumes in the two cell culture chambers were 1.5 ml (apical) and 2.6 ml (basolateral).

Release of Weibel–Palade Body Contents and Drug Treatment of Cells

To stimulate release of vWf from Weibel–Palade bodies, filter-grown cells were rinsed twice with Hank's balanced salt solution, and then culture medium containing the secretagogue was placed in the apical (1.5 ml) and basolateral (2.6 ml) chambers and collected after 10 min. A dilution of the calcium ionophore A23187 (10 µm) (Sigma Chemical Co.) was made from a 10-mM stock solution dissolved in DMSO. When 1 U/ml thrombin (lyophilized human thrombin, 3,250 U/mg; Calbiochem-Behring Corp., La Jolla, CA) was used as the secretagogue, samples were collected into hirudin (Calbiochem-Behring Corp.) to yield a final concentration of 2.5 U/ml.

PMA, used at a concentration of 10 ng/ml, was diluted from a 0.1 mg/ml stock dissolved in DMSO. For studies of the inhibition of release by microtubule-depolymerizing agents, metabolically prelabeled filter-grown cells were incubated for 1 h before and during ionophore stimulation with 1 µM colchicine, 1 µM nocodazol, or 1 µM lumicolchicine (Sigma Chemical Co.) dissolved in culture medium.

Antisera

The preparation and characterization of monospecific antisera against vWf used for immunopurification were previously described (Wagner et al., 1982). Antisera against vWf used for immunofluorescence staining was purchased from Calbiochem-Behring Corp.

Immunofluorescence Staining

Immunofluorescence staining was performed directly on filter-grown cells while in the intact Transwell™ device. Cells were fixed for 20 min in a solution of 3.7% formaldehyde in PBS, then permeabilized for 15 min in 0.5% Triton X-100 in PBS. Immunofluorescence staining using anti-vWf antiserum was then performed as described (Wagner et al., 1982). 1 cm² of the polycarbonate membrane with the attached cell monolayer was removed from the insert with a scalpel blade and mounted in gelvatol under a glass coverslip.

Purification of vWf

Cells were lysed as described previously (Wagner and Marder, 1983) so that the final concentration of ingredients was that of the radioimmunoprecipitation assay buffer (Wagner et al., 1981) used for washing the immunoprecipitate. The cell lysate or culture medium samples were then incubated for 30 min at room temperature with gelatin-Sepharose to remove fibronectin and other proteins that adhere nonspecifically. The gelatin-Sepharose was removed by centrifugation. Protein A-Sepharose CL-4B (30-mg sample) (Sigma Chemical Co.) was preincubated at room temperature for 30 min with 50 µl anti-vWf antiserum before it was added to the samples. The incubation of the samples was for 1.5 h at room temperature. After extensive washing, protein A-Sepharose was boiled in electrophoresis sample buffer (Laemmli, 1970) and the supernatant was analyzed by gel electrophoresis.

Gel Electrophoresis

5% SDS-polyacrylamide gels were prepared as described (Laemmli, 1970). 2% agarose gels were prepared as described (Sporn et al., 1986). Densitometric scanning of autoradiographs was performed and the amount of vWf in the samples was quantitated by determining the area under the peaks.

Iodination of vWf

vWf purified from plasma was a gift from Dr. Philip Fay, University of Rochester, and was iodinated as described (Wagner et al., 1987).

Results

To compare the polarity of the constitutive and regulated secretory pathways for vWf, human umbilical vein endothelial cells were cultured on Transwell™ inserts (see Materials and Methods). Coverage of the growth surface and verification of the endothelial origin of these primary cultures were monitored by immunofluorescence staining using anti-vWf antiserum (Fig. 1). The endothelial cells reached confluence 4–7 d after plating (Fig. 1 a), and contained numerous Weibel–Palade bodies (Fig. 1, a and b). The multimeric composition of vWf immunopurified from metabolically labeled filter-grown endothelial cell lysates and culture medium (Fig. 1) was similar to that seen for endothelial cells cultured on conventional tissue culture plastic (Wagner and Marder, 1983).

Diffusion of vWf between the apical and basolateral chambers was studied using a preparation of iodinated purified
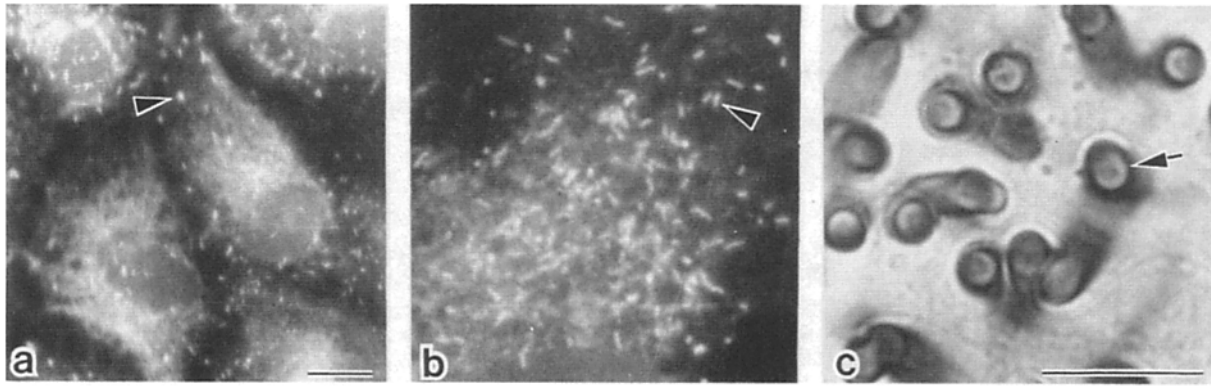
Figure 1. Immunofluorescent staining of endothelial cells cultured on Transwell™ inserts. Cells in *a* (photographed through a 40× objective) and *b* (100× objective) were stained using anti-vWf antiserum. Arrowheads point to Weibel-Palade bodies. *c* is a light microscopic photograph (100× objective), which illustrates the size of the pores (*arrow*) in relation to the size of the cells. Shown also is an autoradiograph of a nonreduced 2% agarose gel showing the multimeric pattern of vWf immunopurified from cell lysates and culture medium of filter-grown cells. Bars, 10 μm.

plasma vWf which contains all multimeric forms (Sporn et al., 1987). All studies were conducted with the endothelial cells on the filter. Diffusion of vWf was found to be concentration and time dependent. When tested over a 100-fold concentration range (0.5-50 μg/ml); 1-9% leakage occurred between chambers (apical to basolateral or basolateral to apical) during a 10-min period. Using 0.5 μg/ml iodinated vWf, the percent diffusion increased to ∼10% in 30 min and to ∼25% in 6 h. To minimize this diffusion, a 10-min collection period was chosen for quantitating the partitioning of vWf originating from each secretory pathway between the apical and basolateral chambers. To determine if the diffusion properties differ for large vs. small multimers, aliquots from the above experiments were electrophoresed, nonreduced, on 2% agarose gels. The multimeric pattern in all samples was indistinguishable (not shown). This rules out the possibility that "settling" of the large vWf multimers into the basolateral chamber occurs.

Constitutive Secretion

First, the polarity of the constitutive secretory pathway of vWf was studied. As a small amount of spontaneous release of vWf from Weibel-Palade bodies is known to occur (Ribes et al., 1987), the experiment was designed to minimize the contribution of vWf from this pool. The intracellular turn-

over of Weibel-Palade bodies is slow (Sinha and Wagner, 1987; Wagner et al., 1986); therefore, short labeling periods of 2 h, an insufficient time for significant labeling of this pool of vWf, were used. Previous results have shown that <5% of the newly synthesized vWf enters the Weibel-Palade bodies during this time (Sporn et al., 1986). After removal of radiolabeled medium, cells were covered with unlabeled medium for 10 min. Then, vWf was immunopurified from both chambers and analyzed by gel electrophoresis. Fig. 2 is a densitometric scan of a nonreduced 2% agarose gel showing the multimeric patterns of the constitutively secreted vWf. vWf secreted into both the apical and basolateral chambers had very similar multimeric compositions, indicating that neither pool had been significantly enriched by spontaneous release of large multimers from the Weibel-Palade bodies. Reduced gel analysis revealed a trace amount of pro-vWf subunits (not shown), which is characteristic of constitutively secreted vWf (Wagner and Marder, 1984). Quantitation by densitometric scanning of the reduced gels showed that approximately equal amounts of vWf were secreted into both the apical and basolateral chambers (Table I), indicating that the constitutive secretory pathway was not polarized.
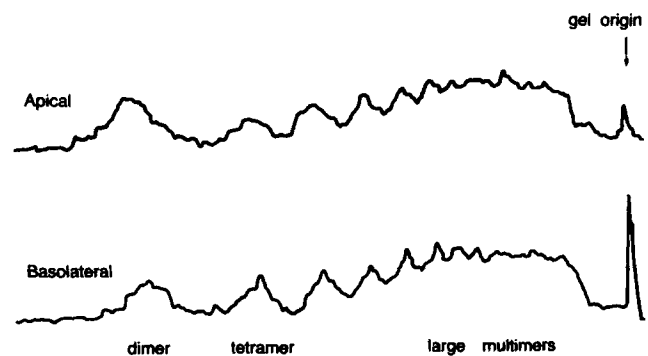

Figure 2. Multimeric pattern of vWf constitutively secreted into the apical and basolateral chambers. After metabolic labeling for 2 h, vWf secreted during a 10-min period without stimulation of cells was collected, immunopurified, and analyzed nonreduced on 2% agarose gels. Since only a small amount of vWf is secreted during this short time, densitometric scans of autoradiographs were chosen to illustrate the multimeric composition of the protein. vWf constitutively secreted into both chambers had similar multimeric pattern, containing all multimeric forms.

Table I. Distribution of Secreted vWf between Apical and Basolateral Chambers

	Apical	Basolateral	SD	n
Constitutive pathway	54	46	3.6	4
Regulated pathway				
A23187	11	89	8.9	5
Thrombin	54	46	7.1	3

Quantitation of metabolically labeled immunopurified vWf was performed by densitometric scanning of autoradiographs of reduced 5% polyacrylamide gels. Percent of total secreted vWf appearing in each chamber is shown.

Regulated Secretion

To study the polarity of stimulated release of vWf from the Weibel–Palade bodies, filter-grown cells were metabolically labeled for 3 d with [^{35}S]cysteine. This assures complete labeling of vWf stored in the Weibel–Palade bodies. After extensive washing of the filters, culture medium was placed in the apical and basolateral chambers and collected after 10 min to determine the amount of constitutive secretion that had occurred. A solution of 10 μM calcium ionophore A23187 in culture medium was then placed in both chambers and collected after 10 min. After immunopurification from the samples, vWf was electrophoresed (both nonreduced and reduced) on gels. Nonreduced gel analysis showed the release of only the largest multimers of vWf, the majority of which appeared in the basolateral chamber (Fig. 3 a). Reduced gel analysis (Fig. 3 b) revealed that, as is characteristic of releasate (Sporn et al., 1986), released vWf contained only mature vWf subunits. It was determined by densitometric scanning of autoradiographs of the reduced gels that an average of 90% of release occurred in the basolateral direction; the remaining 10% appeared in the apical chamber (Table I). When 10 ng/ml PMA was used as the secretagogue,
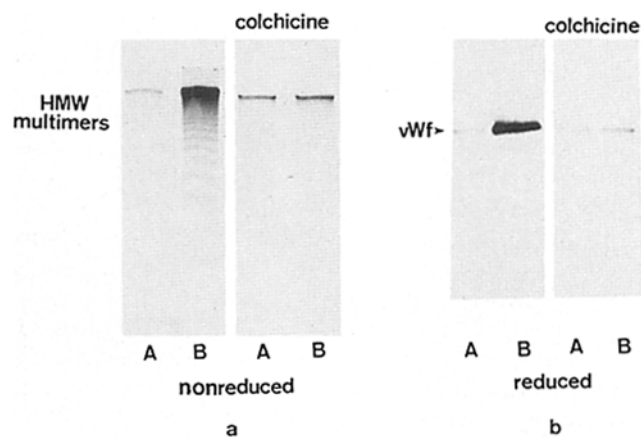
similar results were obtained. On the average, eightfold more vWf was released in the presence of the secretagogue than was secreted constitutively during the collection period.

Thrombin is a physiologic secretagogue that is formed at the site of a vascular injury. Besides causing release of vWf, thrombin has many other effects on endothelial cells (Weksler et al., 1978; Loskutoff, 1979). Exposure to thrombin appears to disrupt the endothelial cell monolayer (Rafelson et al., 1973), making it permeable to dyes (Killackey et al., 1986). In contrast to calcium ionophore and PMA stimulation, when thrombin (1 U/ml) was used as the secretagogue, release appeared to be nonpolarized, with approximately half of the released vWf found in each chamber after a 10-min treatment period (Table I).

The microtubule-depolymerizing drugs nocodazol and colchicine are known to inhibit the stimulated release of vWf from Weibel–Palade bodies (Sinha and Wagner, 1987). To show that the polarized appearance of vWf in the basolateral chamber after stimulation is an inhibitable phenomenon and not the result of cell lysis or shedding of vWf from the extracellular matrix, these experiments were performed in the presence of 1 μM colchicine or nocodazol. The presence of these agents inhibited the basolateral component of release by A23187 by ~90% (Fig. 3). Lumicolchicine (1 μM), an inactive stereoisomer of colchicine, did not cause significant inhibition of release (not shown). After nocodazol treatment, when cells were incubated for 1 h in nocodazol-free medium before ionophore stimulation, large multimers appeared predominantly in the basolateral chamber, indicating that the effect of nocodazol was reversible (not shown).

The Effect of Ammonium Chloride on the Polarity of Release

Ammonium chloride treatment of endothelial cells has been shown to inhibit multimerization of vWf dimers and thereby cause an alteration in the multimeric composition of vWf secreted by the cells (Wagner et al., 1986). A change in the multimeric composition was observed after a 1-h treatment
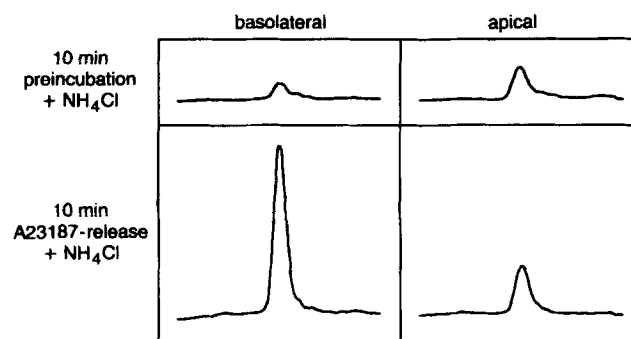

Figure 3. Multimeric and subunit composition of vWf released by the calcium ionophore A23187 (10 μM) into the apical (A) and basolateral (B) chambers. Cells were metabolically labeled for 3 d before ionophore treatment, and immunopurified vWf was analyzed both nonreduced on 2% agarose gels (a) and reduced on 5% polyacrylamide gels (b), autoradiographs of which are shown. The high mol wt (HMW) multimers of vWf (a) composed only of mature vWf subunits (b) appeared predominantly in the basolateral chamber. Pretreatment of cultures with colchicine for 1 h blocked the appearance of vWf in the basolateral chamber after ionophore treatment.

Figure 4. Polarity of vWf release from Weibel–Palade bodies in the presence of ammonium chloride. After metabolic labeling for 3 d, endothelial cells were incubated with 25 mM ammonium chloride for 1 h. Cells were then preincubated for 10 min in the absence of ionophore, followed by 10-min ionophore stimulation. vWf was then immunopurified from samples collected from the apical and basolateral chambers. Ammonium chloride was present during all incubations. Shown are densitometric scans of autoradiographs of reduced 5% polyacrylamide gels. The presence of the weak base did not alter the polarity of Weibel–Palade body release.

period, indicating that this was sufficient time for ammonium chloride to affect the pH of intracellular acidic compartments. A 1-h treatment with ammonium chloride did not cause a noticeable change in the number of Weibel–Palade bodies in the cells as seen by immunofluorescence staining with anti-vWf antiserum (not shown). Therefore, a 1-h treatment period was chosen to study the effect of ammonium chloride on the polarity of release of preexisting Weibel–Palade bodies. After metabolic labeling of filter-grown cells for 3 d, radioactive amino acid–containing medium was removed and the cells were incubated for 1 h in medium containing 25 mM ammonium chloride. Medium containing both 25 mM ammonium chloride and 10 μM A23187 was then placed in both the apical and basolateral chambers. After 10 min, the media were collected and vWf was immunopurified and analyzed by gel electrophoresis followed by densitometric scanning. In the two experiments performed, one of which is shown (Fig. 4), 80 and 100% release of vWf occurred in the basolateral direction, indicating that the presence of ammonium chloride did not alter the polarity of the release process.

Discussion

When endothelial cells cultured on polycarbonate membrane filters were stimulated with calcium ionophore A23187 (Table I) or PMA (not shown), release of vWf from the Weibel–Palade bodies was highly polarized, in that it occurred largely in the basolateral direction. The constitutive pathway of secretion of vWf, however, was not polarized, as approximately equal amounts of vWf were secreted into the apical and basolateral chambers (Table I). Previous work has shown that the two pathways of vWf secretion differ with regard to the molecular forms of the protein originating from them (Sporn et al., 1986), and these results show that the two pathways also differ with regard to polarity. When thrombin was used as a secretagogue for cells grown on filters, approximately equal amounts of vWf were found in the apical and basolateral chambers. Although thrombin-induced release may be nonpolarized, it is more likely that thrombin has other effects on endothelial cells that cause the apparent alteration in polarity. For example, the documented rapid "contraction" of endothelial cells and the resulting disruption of the endothelial cell monolayer by thrombin as well as histamine (Rafelson et al., 1973; Laposata et al., 1983) could allow a rapid escape of vWf from under the cells.

Although diffusion of vWf between chambers was kept to a minimum by keeping treatment times down to 10 min, the small amount (10%) of vWf in the apical chamber after release with the calcium ionophore or PMA could have resulted from such a process. Alternatively, a small amount of release could have occurred directly into the apical compartment. Electron micrographs of unstimulated endothelial cells of rabbit lung show Weibel–Palade body fusion with the apical plasma membrane (McNiff and Gil, 1983); however, such spontaneous release may not exhibit the same polarity as stimulated release. It is also possible that the transendothelial transport system (Simionescu, 1981) may carry some of the basolaterally released vWf to the apical surface. When cells are grown on solid supports vWf appears rapidly, free in solution, in the culture medium (Loesberg et al., 1983; Sporn et al., 1986). In contrast, for cells grown on

filters, a large basolateral reservoir exists which may effectively dilute the protein before it is transported across the endothelium to the apical chamber. The increase in plasma levels of vWf induced by treatment of patients with the vasopressin analogue 1-deamino-8-D-arginine vasopressin is believed to follow release of vWf from Weibel–Palade body stores (Handin and Wagner, 1988). If release occurs predominantly in the basolateral direction in the vessel wall, as it does under our experimental conditions the origin of this increased plasma concentration may reflect only a small percent of the total released vWf or the existence of transendothelial transport.

It is not known what cellular machinery is responsible for polarized secretion. It has been shown for other cell types that special membrane domains exist with which certain vesicle types fuse (Kelly, 1985). Possibly, the Weibel–Palade bodies fuse preferentially with membrane domains found on the basolateral cell surface, whereas constitutive vesicles containing vWf fuse with the plasma membrane at random.

The cellular cytoskeleton may be involved in directing the polarized delivery of Weibel–Palade bodies to the cell surface. Although constitutive vWf secretion is not affected, the microtubule-depolymerizing agents colchine and nocodazol were shown, in a previous study, to inhibit stimulated release of Weibel–Palade body contents (Sinha and Wagner, 1987) and, in the present study, to nearly completely inhibit polarized release. Depolymerization of microtubules has also been shown to inhibit protein secretion in other systems (Antoine et al., 1980; Hall, 1984; Howell and Tyhurst, 1982; Redman et al., 1981). The situation that exists for membrane protein transport, however, appears to be different. The rate and extent of VSV G protein surface expression in virus-infected fibroblasts is not altered due to microtubular disruption (Rogalski et al., 1984), and conflicting reports of the effect of these agents on the polarity of viral protein transport exist. Rogalski et al. (1984) suggested that the polarity of VSV G protein surface expression is altered because of microtubular disruption. Rindler et al. (1987) observed no alteration in polarity of VSV G protein surface expression with microtubular disruption, but did observe altered polarity of influenza hemagglutinin surface expression. Salas et al. (1986) have reported no effect on polarity of VSV and influenza virus budding and little effect on the surface distribution of their envelop proteins when microtubules are altered. Microtubules may provide "tracks" for movement of vesicles (Schnapp et al., 1985) involved in regulated protein secretion, but may not be involved in transport of vesicles carrying plasma membrane glycoproteins.

To understand the nature of the receptor–sorting signal interaction, recent studies of Caplan et al. (1987) have used the weak base ammonium chloride which raises the pH of intracellular acidic compartments (Maxfield, 1982; Dean et al., 1984; Poole and Ohkuma, 1981). Since ammonium chloride disrupted polarized delivery of basement membrane components to the MDCK cell surface, the authors hypothesized that a weak base disrupts receptor–sorting signal interactions which may be dependent on acidic pH. The possibility that ammonium chloride exerts its effect by disrupting vesicular traffic rather than receptor–signal interaction, however, remained to be ruled out. In our experiments, the protein sorting (i.e., deposition of vWf in the Weibel–Palade bodies) occurred before treatment of the cells with ammonium chlo-

ride. In this case, ammonium chloride had no measurable effect on the polarized release from the Weibel-Palade bodies, making randomization of vesicular traffic by the weak base unlikely. We have shown previously that ammonium chloride does interfere with the targeting of vWf into the Weibel-Palade bodies (Wagner et al., 1986). It is likely that the information directing the polarity of secretion is a component of the secretory vesicle membrane, and that changes in intracellular pH do not affect the vesicle transport process.

A platelet-derived growth factor-like chemotactic factor which induces chemotaxis and cell migration in fibroblasts and smooth muscle cells was shown also to be secreted predominantly in the basolateral direction by cultured bovine aortic endothelial cells (Zerwes and Risau, 1987). Demonstration that this factor is abluminally released supports the hypothesis that it functions to recruit vascular wall cells during morphogenesis or disease. From the result we obtained on the stimulated release of vWf from endothelial cells, we can also speculate on the role of released vWf in the vessel wall. Our results suggest that stimulation of endothelial cells with secretagogues that do not cause cell retraction results in basolateral release of vWf. Other physiologic secretagogues that are known to disrupt the integrity of the endothelial cell monolayer, such as thrombin (Rafelson et al., 1973) and fibrin (Kadish et al., 1979), would cause either the immediate appearance of some of the large multimers in surrounding blood or the exposure of newly incorporated basement membrane vWf to the blood where it could contribute to the formation of a platelet plug. This pool of highly adhesive released vWf could also function to better reattach the injured endothelium to the vessel wall by binding the transmembrane vWf receptor to the vitronectin receptor (Charo et al., 1987; Cheresh, 1987) which is present in the adhesion plaques of endothelial cells (Dejana et al., 1988). Released vWf may also aid in the migration of endothelial cells into the wounded area, just as the addition of fibronectin to normal and transformed cells has been shown to stimulate the migration of fibroblasts (Ali and Hynes, 1978). Other evidence which supports the notion that vWf functions in events other than platelet adhesion is the fact that endothelial cells that line lymphatic vessels (which do not normally come in contact with platelets) were reported to synthesize vWf and to contain Weibel-Palade bodies (Tabuchi and Yamamoto, 1974).

In contrast to the regulated secretion of vWf, constitutive secretion was not polarized. This pool of predominantly small multimers may provide a continuous supply of vWf to the basement membrane where it could be covalently cross-linked to other basement membrane constituents (Bockenstedt et al., 1986). In addition, these small multimers secreted constitutively into the bloodstream are suitable to serve as protective carrier molecules for factor VIII, the antihemophilic factor.

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