Dynamics and Plasticity of Weibel-Palade Bodies in Endothelial Cells

Mariska G. Rondaij, Ruben Bierings, Astrid Kragt, Jan A. van Mourik, Jan Voorberg

Abstract—Agonist-induced release of endothelial cell specific storage granules, designated Weibel-Palade bodies (WPBs), provides the endothelium with the ability to rapidly respond to changes in its micro-environment. Originally being defined as an intracellular storage pool for von Willebrand factor (VWF), it has recently been shown that an increasing number of other components, including P-selectin, interleukin (IL)-8, eotaxin-3, endothelin-1, and angiopoietin-2, is present within this subcellular organelle, implicating a role for WPB exocytosis in inflammation, hemostasis, regulation of vascular tone and angiogenesis. Recent studies emphasize that WPBs provide a dynamic storage compartment whose contents can be regulated depending on the presence of inflammatory mediators in the vascular micro-environment. Additionally, release of WPBs is tightly regulated and feedback mechanisms have been identified that prevent excessive release of bioactive components from this subcellular organelle. The ability to regulate both contents and exocytosis of WPBs endows these endothelial cell specific organelles with a remarkable plasticity. This is most likely needed to allow for controlled delivery of bioactive components into the circulation on vascular perturbation. (Arterioscler Thromb Vasc Biol. 2006;26:1002-1007.)

Key Words: endothelial cells ■ exocytosis ■ hemostasis ■ inflammation ■ Weibel-Palade body

Endothelial cells lining the vasculature provide a tightly regulated barrier that regulates a number of physiological processes, including extravasation of leukocytes to the underlying tissues, neovascularization in response to vascular injury, vascular tone, and hemostasis. Over the past few years it has been appreciated that endothelial cells, depending on their location within the vasculature have distinct gene expression profiles. Different vascular beds are thereby equipped with unique properties. Additional diversity is generated by rearrangement of gene expression patterns in response to inflammatory mediators like tumor necrosis factor-α or hemodynamic changes. Adaptation to physiological and pathological changes by modulation of gene expression requires at least several hours, to allow for transcription/translation and transport of proteins to the exterior or surface of the cell. In specific instances a more rapid response to vascular perturbation is necessary and endothelial cells deal with incoming challenges by immediate recruitment of bioactive components from intracellular storage pools. Perhaps the best characterized intracellular storage pools within endothelial cells are so-called Weibel-Palade bodies (WPBs), rod-shaped, elongated structures that appear like “chocolate sprinkles” in the cytoplasm (Figure 1). In the original electron microscopic work of Ewald R. Weibel and George E. Palade the dimension of these organelles were defined (width 0.1 μm and up to 3 μm in length) and the tubular nature of this organelle, that was especially apparent in transverse sections, was noted (see Figure 1). In this brief review we discuss recent data on the increasing list of proteins that reside in WPBs and provide insight into the dynamics and regulation of exocytosis of this subcellular organelle.

See cover

Biogenesis of WPBs

The major constituent of WPBs is the multimeric protein von Willebrand factor (VWF). VWF is required for correct hemostasis through its role in platelet adhesion at sites of vascular injury. Several lines of evidence support the idea that VWF is the driving force behind the biogenesis of these organelles. Expression of VWF in nonendothelial cells results in the formation of VWF-containing, rod-shaped organelles that closely resemble WPBs. In agreement with these findings in endothelial cells derived from dogs with severe, type 3 von Willebrand disease that lack WPBs biogenesis of these organelles is restored on expression of VWF. The requirements for VWF-induced formation of WPBs have been studied by several groups and have previously been discussed in 2 separate reviews and are addressed extensively here. Two recent reports have shed light on the mechanism of formation of WPBs from the trans-Golgi

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network. Liu-Roberts et al showed the presence of clathrin coats on nascent WPBs and revealed that the clathrin-associated adaptor protein complex AP-1 is essential for the formation of WPBs. They speculated that a cytoplasmic coat allows for the formation of the elongated, rod-shaped structure of WPBs. Another report revealed a putative regulator of the typical shape of WPBs. Overexpression of an active variant of the small GTP binding protein Rab3D results in bigger and more spherical WPBs. Rab3D has previously been suggested to interfere with homotypic fusion of secretory granules during their maturation. Remarkably, overexpression of an inactive variant of Rab3D resulted in the absence of WPBs suggesting that Rab3D is involved in biogenesis of WPBs. The reports on AP-1 and Rab3D highlight ongoing work which will hopefully provide more insight into the remarkable architecture of WPBs. In view of the crucial role of VWF in the biogenesis of this subcellular organelle, these studies are likely to also generate novel insight into the macromolecular organization of VWF within WPBs.

Landlords and Tenants: Residents of WPBs

Over the past few years it has been appreciated that multiple components are costored with VWF in WPBs. The growing list of components that are present within this subcellular organelle suggests a role for regulated release of WPB in inflammation, hemostasis, hemodynamics and angiogenesis (Table 1). It equips the endothelium with the ability to rapidly respond to changes within its micro-environment. Perturbation of endothelial cells and subsequent exocytosis of WPBs may initiate hemostasis (VWF), induce vasoconstriction to prevent unnecessary loss of blood components (endothelin-1 and its converting enzyme), regulate inflammatory responses (P-selectin, IL-8 (IL-8), angiopoietin-2 (Ang-2), CD63, α1,3-fucosyltransferase VI, osteoprotegerin (OPG) and direct fibrinolysis (tissue-type plasminogen activator [tPA]; Table 1). The remarkable diversity of effectors present within a single organelle that can be recruited by a single agonist is surprising and, at first sight, might even seem hazardous. It has been well-established that even mild exercise results in a rise in plasma levels of VWF that most likely originate from WPBs. Excessive release of for instance inflammatory mediators is undesirable under these conditions. Presently, 2 mechanisms have been defined that regulate release of bioactive components present within WPBs under quiescent conditions. Several studies have provided evidence for the existence of different subsets of WPBs that apart from VWF do not contain the same set of additional constituents. A clear example is the chemotactic cytokine IL-8. This cytokine is stored in WPBs only after induction of its synthesis by inflammatory mediators such as IL-1β, thus providing a rapidly releasable pool of IL-8, which is independent of de novo synthesis. On overnight incubation of human umbilical cord endothelial cells with IL-1β, IL-8 was not detected in all WPBs present within endothelial cells. WPBs that showed no immunoreactivity for IL-8 were probably already formed before cells were exposed to IL-1β. This equips the cells with temporally divided WPB subtypes. Similarly, eotaxin-3 is only routed to WPBs following stimulation of endothelial cells with IL-4. Also, tPA was found in only a part of the VWF-positive WPBs. The WPB distribution of Ang-2 was even more intriguing because, although both P-selectin and Ang-2 are sorted to WPBs, no colocalization between these proteins was observed. P-selectin has been reported to be internalized following surface expression and be recycled back to WPBs.

Table 1. Contents of Weibel-Palade Bodies

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWF/7</td>
<td>Hemostasis</td>
</tr>
<tr>
<td>P-selectin/17,18</td>
<td>Inflammation, leukocyte adhesion</td>
</tr>
<tr>
<td>Interleukin 8 (IL-8)/19,20</td>
<td>Inflammation, granulocyte adhesion/migration</td>
</tr>
<tr>
<td>Eotaxin-3/21</td>
<td>Inflammation, eosinophil recruitment/migration</td>
</tr>
<tr>
<td>Calcitonin gene related peptide/22</td>
<td>Vasoconstriction</td>
</tr>
<tr>
<td>Endothelin/22,23</td>
<td>Vasoconstriction</td>
</tr>
<tr>
<td>Endothelin converting enzyme/24</td>
<td>Vasoconstriction</td>
</tr>
<tr>
<td>CD63/α1,3-fucosyltransferase VI/26</td>
<td>Cell adhesion/migration</td>
</tr>
<tr>
<td>Tissue-type plasminogen activator (tPA)/28</td>
<td>Membrane glycosylation</td>
</tr>
<tr>
<td>Angiopoietin-2/29,30</td>
<td>Fibrinolysis</td>
</tr>
<tr>
<td>Osteoprotegerin/31</td>
<td>Inflammation, vascular homeostasis</td>
</tr>
</tbody>
</table>

Figure 1. Morphology of Weibel-Palade bodies. A, Electron micrograph of WPB induced by VWF expression in HEK293 cells showing the parallel alignment of internal striations. B, Transverse section of WPB reveals the tubular composition of WPB (courtesy of Dr E. R. Weibel). C, Immunostaining with anti-VWF antiserum reveals budding of newly formed WPB (large arrow) from the trans Golgi network (G). The limiting membrane between WPB and trans-Golgi network is indicated by a small arrow. D, Distribution of WPBs in a resting endothelial cell expressing GFP-VWF. A, Reprinted from reference 10 with permission; copyright American Society of Hematology. C, Reprinted from reference 9 with permission. B, Kindly provided by Dr Ewald Weibel.
for reuse.\textsuperscript{26,34} Trafficking of P-selectin from recycling endosomes to a subset of WPBs that does not contain Ang-2 has been proposed as a possible mechanism for the mutually exclusive presence of these proteins in WPBs.\textsuperscript{29} Together these data provide evidence for the dynamic regulation of WPB contents. Potentially, selective release of subsets of WPBs would provide a means to regulate the release of bioactive components from endothelial cells.

An additional mechanism to modulate release of WPBs has recently been uncovered. In the presence of agonists that raise intracellular cAMP clustering of WPBs at a perinuclear region is observed.\textsuperscript{35–37} This phenomenon most likely prevents excessive release of WPB constituents by this class of agonists. In addition, clustering allows for the selective exclusion of subsets of WPBs from exocytosis. Real-time analysis of WPB trafficking shows the presence of near-immobile WPBs suggestive of morphologically docked vesicles.\textsuperscript{36,38} Moreover, in unstimulated endothelial cells, a small subset of the WPBs was found to be associated with the actin cortex.\textsuperscript{37} These vesicles are assumed to constitute a readily releasable pool (RRP) of WPBs. The larger, remaining, microtubule-associated WPB pool can be viewed as a dynamic stockpile from which WPBs are either recruited to replenish the RRP or, are recruited to the microtubule organizing center on stimulation with cAMP raising agonists.\textsuperscript{35,36,39} Interestingly, the small GTPase Rab27 has been localized to WPBs.\textsuperscript{40} In melanocytes Rab27 has been implicated in transfer of melanosomes from microtubules to actin filaments.\textsuperscript{41} Based on these findings it is likely that Rab27 is involved in intracellular trafficking of WPBs although its precise role remains to be established.

### Dynamics and Regulation of Exocytosis of WPBs

WPBs are released from endothelial cells in response to a large number of secretagogues such as thrombin,\textsuperscript{42} histamine,\textsuperscript{43} peptido-leukotrienes,\textsuperscript{44} complement components C5a and C5b-9,\textsuperscript{45,46} superoxide anion,\textsuperscript{47} vascular endothelial growth factor (VEGF),\textsuperscript{48} sphingosine 1-phosphate,\textsuperscript{49} ceramide,\textsuperscript{50} purine nucleotides,\textsuperscript{51} serotonin,\textsuperscript{52} epinephrine,\textsuperscript{53} and vasopressin.\textsuperscript{54} These agonists of VWF secretion can be divided into 2 distinct groups, those that act by elevating intracellular Ca\textsuperscript{2+} levels and those that act by raising cAMP levels in the cell (Table 2). In case of vascular damage, thrombin, one of the best studied Ca\textsuperscript{2+}-raising agonists of VWF secretion, induces a rapid, local response leading to exocytosis of most of the WPBs present in the cell.\textsuperscript{35} Several studies have shown that the responses to elevated Ca\textsuperscript{2+} levels are most likely mediated by the Ca\textsuperscript{2+}-binding protein calmodulin.\textsuperscript{55,56} VWF secretion in response to cAMP-raising agonists was also shown to be of physiological importance because the concentration of VWF in blood is raised in response to epinephrine,\textsuperscript{57} which is released, for example, during physical exercise. Similarly, the vasopressin analogue desmopressin is used in a subset of VWD patients to raise plasma VWF levels.\textsuperscript{58} In vitro, cAMP-mediated VWF secretion could be blocked by the inhibition of protein kinase A, the most common effector of cAMP signaling.\textsuperscript{53,54} These data indicate that 2 distinct sets of stimuli exist that induce VWF release through different pathways. Both Ca\textsuperscript{2+}-raising and cAMP-raising agonists display agonist-specific patterns of cytoskeletal remodeling that have pronounced effects on endothelial cell barrier function.\textsuperscript{35} Incubation with thrombin and histamine results in activation of the small GTPase RhoA, which results in stress fiber formation and loss of endothelial cell barrier function by disassembly of tight and adherens junctions.\textsuperscript{59,60} In contrast, cAMP-raising agonist like epinephrine and vasopressin have recently been shown to result in the activation of the small GTPase Rap1, which promotes VE-cadherin mediated cell-cell contact and improves barrier function of endothelial cells.\textsuperscript{61–63} It is of interest that WPB exocytosis is induced by agonists with opposing effects on endothelial cell barrier function (Figure 2). We anticipate that release of pro-inflammatory mediators like P-selectin and IL-8 after stimulation with cAMP-raising agonists is neutralized by increased endothelial barrier function. In contrast, Ca\textsuperscript{2+}-raising agonist like thrombin and histamine are expected to induce a much more vigorous response in which release of pro-inflammatory mediators from WPBs attracts leukocytes that can now rapidly infiltrate underlying tissues by virtue of the strongly reduced endothelial barrier function.

### Conclusions and Remaining Issues

Originally being defined in aortic endothelial cells, the distribution of WPBs along the vascular tree is heterogeneous.\textsuperscript{64} Especially high numbers of WPBs were found in the pulmonary artery, a finding consistent with tissue distribution of VWF.\textsuperscript{65} Recently, it has been shown that the expression profiles of endothelial cells from different vascular origin have many similarities but also display remarkable differences.\textsuperscript{2} In view of these findings it is anticipated that the content of WPB will differ among different vascular beds. Another issue involves the physiological importance of storage of bioactive components within WPBs. Storage of IL-8 and eotaxin-3 may serve as a rapid “first aid” delivery of these inflammatory compounds after vascular perturbation.\textsuperscript{19–21} The physiological significance of storage within WPB has only been addressed for P-selectin. An elegant study docu-
not only into the biogenesis of these elegantly shaped organelles but also on the requirements for entry into this versatile storage compartment within endothelial cells.

Acknowledgments

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