Tuning the SNAREs that regulate Weibel-Palade body exocytosis
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Summary

Endothelial cells form the inner lining of the vessel wall and provide a barrier between circulating blood and the underlying tissue. The endothelium participates in maintenance of vascular homeostasis and facilitates rapid responses to environmental changes such as inflammation or vascular damage. A cocktail of bioactive components participating in these processes are stored in endothelial-specific storage organelles, designated Weibel-Palade bodies (WPBs). The main component of WPBs is von Willebrand factor (VWF). VWF functions in arrest of bleeding by capturing platelets thereby promoting thrombus formation at sites of vascular injury. Furthermore, VWF acts as a carrier for coagulation factor VIII. Several other mediators of hemostasis, inflammation and angiogenesis are co-stored with VWF in WPBs and are secreted into the vascular lumen upon WPB exocytosis. In this thesis we aim to obtain a better understanding of the composition of WPBs and the molecular mechanisms that regulate the release of WPB components into the bloodstream.

In Chapter 1 we discuss the physiological role of WPBs and describe functions of known WPB residents. Furthermore we provide an overview of our current knowledge on WPB biogenesis and highlight molecular regulators of WPB exocytosis. To obtain a better insight into the physiological importance of WPBs we explored the content of WPBs using an unbiased proteomic analysis of subcellular fractions that are enriched for WPBs (Chapter 2). In this screen we identified the angiogenic compound insulin-like growth factor binding protein 7 (IGFBP7) as a novel bona fide WPB component. This result emphasizes the prominent role that WPBs play in the regulation of angiogenesis.

In the next chapters we focus on molecular mechanisms of WPB exocytosis, in particular the contribution of SNARE and SNARE accessory proteins. In Chapter 3 we explored the mechanism by which Slp4-a regulates WPB exocytosis. To this end, we used a proteomic approach to identify interactors of Slp4-a in endothelial cells and revealed syntaxin binding protein 1 (STXBP1), syntaxin-2 and syntaxin-3 as binding partners of Slp4-a. SiRNA mediated silencing of STXBP1 protein expression in human umbilical vein endothelial cells (HUVECs) resulted in decreased secretagogue-evoked VWF secretion. Furthermore, blood outgrowth endothelial cells (BOECs) derived from an early infantile epileptic encephalopathy type 4 (EIEE4) patient carrying a heterozygous mutation in the gene encoding STXBP1 displayed impaired hormone-evoked VWF secretion. These data identify STXBP1 as a
critical positive regulator of WPB exocytosis. In this study we also showed that STXBP1 binds to syntaxin-2 and syntaxin-3 in a mutually exclusive manner. In Chapter 4 we investigated the subcellular localization of syntaxin-2 and syntaxin-3. We showed that syntaxin-2 localizes on the plasma membrane of endothelial cells and we identified syntaxin-3 as a novel WPB-associated SNARE protein. We subsequently investigated the role of syntaxin-3 in WPB dynamics and demonstrated that silencing of syntaxin-3 protein expression resulted in an increase in intracellular VWF levels, probably due to a decreased basal turnover of WPBs. These results position syntaxin-3 as a regulator of basal VWF secretion.

Genome wide association studies have linked genetic variation in the gene encoding for syntaxin binding protein 5 (STXBP5) to plasma VWF levels. In Chapter 5 we analyzed the role of STXBP5 in WPB exocytosis. Downregulation of STXBP5 expression demonstrated that STXBP5 acts as a negative regulator of VWF secretion. Moreover, a decrease in hormone-evoked VWF secretion was observed upon expression of the carboxyl-terminal VAMP-like domain of STXBP5. In addition, our data suggest that the inhibitory role of STXBP5 is mediated by an association of STXBP5 with syntaxin-3 and syntaxin-4.

Finally, in Chapter 6 we discuss the major findings of this thesis and propose possible molecular mechanisms for the novel regulators of VWF secretion identified in this study.