The decrease of deformability of glutaraldehyde-pretreated endothelial cells has been used by some authors as evidence for the capacity of endothelium to respond to mechanical stimulation[9]. In our experiments, glutaraldehyde pretreatment of the vascular bed significantly decreased the levels of both RH and of the relaxation of the test segment of femoral artery (fig.), i.e. the effect was accompanied by substantial inhibition of the release of vasodilators into the blood. However, the response to acetylcholine against the background of dimerized glutaraldehyde remained unchanged. In control experiments, the shift of the response to acetylcholine in the bed of the femoral artery was $+ 164 \pm 10.0\%$ of initial blood flow level, compared with $+ 122.0 \pm 12.2\%$ following glutaraldehyde pretreatment. It may be assumed therefore that the response to chemical stimuli was not affected by the pretreatment.

In conclusion, RH is largely a result of the release of biologically active vasodilators by the endothelial cells. The stimulus which initiates the occlusion-related synthesis and release of such vasodilators by the endothelium has been shown to be the fall of intravascular pressure. The capacity of endothelium to respond with the release of dilator factor as a flow-dependent vascular reaction is also well known[16,17]. Undoubtedly, this does occur when the blood flow is restored after occlusion. However, the fact that the acceleration of blood flow does not depend on the duration of occlusion, whereas the degree of hyperemia is strictly dependent on the latter, may be used as an argument against such a mechanism for the initiation of endothelium secretory activity and the subsequent development of RH.

In the development of vascular reactions and of RH in particular, a special role has been attributed to adenosine[10]. Apart from the direct effect of adenosine on smooth muscle cells, the possible involvement of EDRF in the vasodilatory effect of adenosine should not be excluded. Such a possibility is supported by data on the participation of the endothelium in the adenosine effect[18,19].

The data presented testify to the involvement of endothelium in the development of RH, which can be achieved as a result of various factors which stimulate the synthetic activity of the endothelium.

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Effect of vasoactive amines on Weibel-Palade bodies in capillary endothelial cells

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Abstract. The presence and distribution of Weibel-Palade bodies in stomach and colonic mucosal microvessels after the administration of vasoactive amines (serotonin and histamine), the serotonin depletor reserpine, and the von Willebrand factor secretagogue thrombin, was studied by transmission electron microscopy. These agents elevated the number of Weibel-Palade bodies in all microvascular endothelial cells and especially in capillaries. It is concluded that vasoactive amines enhance the synthesis and secretion of large von Willebrand protein multimers by endothelial cells.
Key words. Capillary; endothelial cell; mucosa; Weibel-Palade body; von Willebrand factor; electron microscopy; rat.

The von Willebrand factor (vWF) mediates the attachment of platelets to the subendothelium [13]. It is synthesized in endothelial cells and megakaryocytes. The largest multimeric forms of vWF are stored in the endothelium-specific Weibel-Palade bodies (WPb)[16]. It is secreted from there mainly upon stimulation by various secretagogues including thrombin [7]. This process is called the regulated pathway of release, and differs from the constitutive pathway. Most of the existing data come from experiments performed on cultured endothelial cells; in vivo studies are relatively rare. The aim of this study was to follow in vivo changes in WPb number in microvascular endothelium after exposure to vasoactive amines like serotonin (5-HT) and histamine.

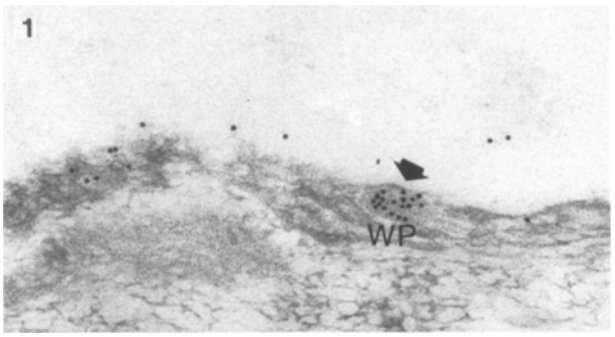

Figure 1. Immunocytochemical localization of von Willebrand factor in Weibel-Palade bodies (WP) and in the cytoplasm of a capillary endothelial cell of colonic mucosa. × 54000.

Materials and methods

Experiments were carried out on adult male Wistar rats. Serotonin creatinine-sulphate (150 µg/100 g) was administered intraperitoneally. Samples were taken from the wall of the stomach and large intestine 15 and 60 min after injection. In a second group, 5-HT (100 µg diluted in saline) was infused via the left ventricle by a perfusion-pump for 60 s. Samples were taken immediately after perfusion. Histamine dihydrochloride was infused through the femoral vein in a single dose at three concentrations: 3 µg/100 g, 125 µg/100 g, and 250 µg/100 g, and the animals were sacrificed after 90 and 210 s. Reserpine (Fluka), dissolved in a solution of ethanol-acetic acid-distilled water (20:1:1), was injected intraperitoneally in a single dose of 2.5 mg/kg. Samples were taken 19 h after the application of the drug. Thrombin (1 U/ml) was infused after washing off the circulating blood and allowed to circulate for 5, 30 and 60 min.

All samples from control and experimental animals were then processed using routine methods for conventional transmission electron microscopy. For the localization of vWF in microvascular endothelial cells, grid-mounted ultrathin sections, embedded in LR White resin (London Resin Co., London UK)[10], were immunostained using the post-embedding immunogold staining method [2]. Antibody to human factor VIII-related antigen (DAKO Ltd) was used at a dilution of 1:1000 overnight at 4 °C. This antibody was kindly donated by S. Van Noorden from the Department of Histopathology, RPMS, London. It was also found that it could be used to localize rat vWF by immunofluorescence. Goat anti-rabbit 10 nm gold probe (Amersham International, UK) was used as a second layer. Morphometric analysis was made on conventional transmission electron micrographs of capillary profiles in mucosal microvessels. Endothelial surface was measured using a Maho-N:60 planimeter. The measurement was made on the peripheral extranuclear zone of the capillary endothelium. WPb counts per µm^2 were calculated in control and experimental groups. Results are expressed as the means and the standard errors of the means of the actual calculations. For each item in the table the significance of the difference between the control and each experimental group was estimated using a t-test.

Results and discussion

Electron-microscopical examination revealed that at the start of the experiment vWF-immunoreactivity was non-uniformly distributed in the cytoplasm and in WPb (fig. 1), which were extremely rare in the mucosal capillary endothelium (table). WPb were only found in 23% of the endothelial profiles, and those found were single ones. 15 min after the intraperitoneal administration of 5-HT, the number of WPb increased in all microvessels. In capillary endothelial cells they were found in groups of 2–3 in their usual localization in the paranuclear and peripheral zones of the cells. 60 min after serotonin injection the results resembled those in the control group. A substantial rise in WPb counts was found after infusion of 5-HT. In 83% of the capillary profiles, 1–6 WPb were found. They were often observed in a 'secretory' position facing the cellular membrane. Histamine led to a relative rise in WPb number in capillary and postcapillary (fig. 2, table) endothelium in a dose-dependent manner (peak at 250 µg/100 g/90 s). The WPb were often local-

Distribution of WPb per µm^2 in capillary endothelium. N - number of capillary profiles counted. Student's t-test.

Vasoactive substance	Control	Histamine	Serotonin i.v.	Reserpine	Thrombin
Number of WPb/µm^2	0.24	2.48	2.25	4.47	3.62
Standard deviation	0.10	0.24	0.29	0.31	0.46
P		0.05	0.05	0.05	0.05
N	30	30	30	30	30

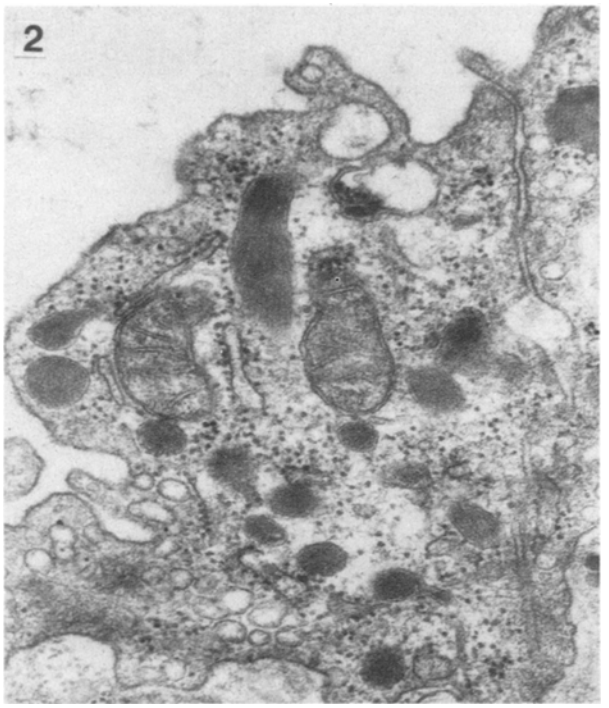

Figure 2. Accumulation of Weibel-Palade bodies in stomach mucosal venous-limb capillary after histamine treatment (250 µg/100 g/90 s). Note the association of these organelles with the rough endoplasmic reticulum. × 50 000.

ized at the interendothelial junctional zone, which normally is extremely rare. Local dilatations of the rough endoplasmic reticulum, especially in stomach vessels (fig. 2), were also observed. Reserpine caused a considerable rise in the number of WPbs (table). They were found in practically all sections and their number per endothelial profile varied from 2 to 9. With thrombin, 30 min after infusion, there was a rise in the WPb count similar to that caused by reserpine and infused 5-HT (table). 60 min after infusion there was a reduction in the number of WPb.

Our results show that vasoactive amines like serotonin and histamine cause a relative increase in the number of Weibel-Palade bodies, which is most clearly evident in capillary endothelial cells because under normal conditions the number there is low. Reserpine causes a similar rise, probably by depleting platelet and tissue monoamine stores[4, 9]. The fact that 5-HT infusion greatly resembled reserpine action confirms our belief in a possible direct action on the endothelium in vivo.

Elevation of WPb number could be caused either by enhanced synthesis and secretion of large vWF-multimers, or by a block of their exocytosis, with a resultant intracellular WPb accumulation. The thrombin-induced rise in WPb content, however, leads us to conclude that vasoactive monoamines most probably also stimulate secretion of vWF stored in WPb. In an elegant in vitro immunohistochemical study, Sporn et al.[14] proved this to be the case with various secretagogues like PMA, A23 187 and to some extent thrombin. An increase in the number of these organelles could also result from a relatively rapid shift of a pre-formed intracellular pool of large vWF multimers to newly-forming WPb. Vasopressin, serotonin and histamine in vivo elevate the plasma level of vWF[5, 12], and the level is also increased in various diseases like arthritis and encephalitis[3, 11], while the number of WPb increases in glaucoma[1], rheumatism[6], Behchet disease[8] and atherosclerosis[15]. In all these diseases vasculitis also occurs. We conclude that examination of the relative distribution of WPb in biopsy specimens, together with changes in the level of von Willebrand factor in the plasma could help in the evaluation at coagulation disturbances.

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