DDAVP and Epinephrine-Induced Changes in the Localization of von Willebrand Factor Antigen in Endothelial Cells of Human Oral Mucosa

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A peroxidase-labeled antibody technique revealed von Willebrand factor antigen (vWF:Ag) in rough endoplasmic reticulum (rER), the perinuclear region, and the cytoplasmic vesicles of capillary endothelial cells in oral mucosa. After administration of epinephrine or 1-deamino-8-D-arginine vasopressin (DDAVP), the localization of vWF:Ag was shown to have changed to the basement membrane and the surrounding interstitium. This change of vWF:Ag localization induced by epinephrine and DDAVP may play a role in the adhesion of platelets to subendothelium following endothelial injury during surgery and may be an unknown hemostatic effect of these drugs.

The von Willebrand factor antigen (vWF:Ag) is synthesized by human endothelial cells and megakaryocytes. Epinephrine, nicotinic acid, vasopressin, and other drugs affecting vascular motility increase factor VIII coagulant activity (VIII:C) and plasma vWF:Ag levels in humans. Especially 1-deamino-8-D-arginine vasopressin (DDAVP), a synthetic analogue of vasopressin, is hemostatically effective in patients with mild and moderate hemophilia A and certain types of von Willebrand’s disease (vWD). Elevation of plasma vWF:Ag induced by epinephrine or DDAVP may be caused by release of vWF from endothelial cell storage sites. However, until now no morphological evidence for this release mechanism has been found. We aimed to clarify this mechanism by using a peroxidase-labeled antibody technique and observing the vasoactive drug-induced changes in localization of vWF:Ag in the endothelial cells of oral mucosa at the electronmicroscopic level.

MATERIALS AND METHODS

Three biopsy specimens were obtained from each of 14 patients. The epinephrine group included five patients (mean age, 6.8 months) with a cleft lip and palate, and the DDAVP group included four patients (mean age, 35 years) with a variety of oral lesions. In addition, biopsy specimens were obtained from five control patients (mean age, 31.4 years) without treatment by epinephrine or DDAVP.

Tissue specimens. In the epinephrine group, biopsy specimens were obtained from labial or palatal mucosa of each patient, before and 30 and 60 minutes after administration of a local anesthetic containing epinephrine (5.7 to 8.3 μg/kg) during cheiloplasty or palatoplasty after the start of a minor operation (open reduction of fractured mandible, radical operation of the maxillary sinus, extirpation of mandibular cyst, radical operation of maxillary sinus) under general anesthesia. Tissue specimens for gingiva of two patients with severe vWD were used to demonstrate the specificity of the anti-vWF:Ag antiserum. Plasma levels of vWF:Ag in both patients were <1%. Antibodies. Rabbit anti-human vWF:Ag antiserum was obtained from Dako Immunoglobulins (Kyowa Medics, Tokyo). The specificity of this polyclonal antibody was established by immunoelectrophoresis and immunodiffusion in agarose gel. Fab' fragments of γ-globulin fraction were labeled with horseradish peroxidase (HRP) at Tokai University according to the method of Wilson and Nakane. The monospecificity of this HRP-labeled Fab' fragment was confirmed by lack of staining of tissues from two patients with severe vWD. For controls, Fab' fragments of nonimmune rabbit γ-globulins were also labeled with HRP.

Immunocytochemistry. Tissue specimens were immediately fixed in periodate-lysine-4% paraformaldehyde for 6 hours at 4°C, washed in increasing concentrations of sucrose in 0.01 mol/L sodium phosphate buffer pH7.4 (PBS), and embedded in OCT compound (Miles Scientific, IL). Tissue blocks were cut with a cryostat into sections 6-μm thick, which were placed on glass slides coated with egg albumin and dried at room temperature. vWF:Ag was localized by the peroxidase-labeled antibody method as previously described. Sections to be observed by light microscopy were treated with 100% methanol and 0.03% hydrogen peroxide to inactivate endogenous peroxidase. The sections were immersed in 10% nonimmune rabbit serum and reacted overnight with the HRP-labeled antibody at 4°C. Control sections were treated with the HRP-Fab' fragments of nonimmune rabbit γ-globulins. After being washed in PBS, the sections were reacted with 0.25% diaminobenzidine (DAB) solution containing 0.01 mol/L hydrogen peroxide and 10 mmol/L sodium azide and then counterstained with methylgreen.

Sections to be examined by electronmicroscope were handled almost identically, except that the treatment with 100% methanol and 0.03% hydrogen peroxide was omitted. The sections were fixed in 0.5% glutaraldehyde and washed. They were then immersed sequentially in 0.25% DAB solution and in 0.25% DAB solution containing hydrogen peroxide. They were then washed and fixed in 2% osmium tetroxide in PBS for 1 hour, dehydrated in graded ethanol, and embedded in Epon 812. Ultrathin sections, stained with lead citrate, were viewed with a Hitachi H-300 electron microscope.

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Fig 1. Immunohistochemical staining for vWF:Ag in a section from palatal mucosa in epinephrine group. (a) Before administration of epinephrine. vWF:Ag is present in a fine granular pattern in the cytoplasm of the endothelial cells. (b) Control staining before administration of epinephrine. The section reacted with nonimmunized rabbit serum is negative. (c) Thirty minutes after epinephrine administration (5.9 µg/kg). Staining is fainter, and vWF:Ag-positive cytoplasmic granules is sparse and coarse. (d) Sixty minutes after epinephrine administration (5.9 µg/kg). Staining is much weaker. Original magnification x150.

RESULTS

Light microscopy. Reaction products of the HRP-labeled anti-vWF:Ag were always found in a fine granular pattern in the cytoplasm of the endothelial cells before administration of epinephrine or DDAVP (Figs 1A and 2A). Thirty minutes after administration of either drug, the staining was fainter and vWF:Ag-positive cytoplasmic granules were sparse and coarse. Occasionally, vWF:Ag was present on the basement membrane (Figs 1B and 2B). Sixty minutes after administration, staining became much weaker and some endothelial cells were sparsely stained (Figs 1C and 2C). There were no differences in the findings between the epinephrine and DDAVP administration groups. The control stainings were uniformly negative (Fig 1D). In controls, no change of staining occurred 30 or 60 minutes after the start of operation (Fig 3).

Electron microscopy. Immunoelectron microscopy corroborated the light microscopic findings. There was almost no difference in the findings between the epinephrine and DDAVP groups. Before administration of epinephrine or DDAVP, vWF:Ag was localized in the protein-synthetic organelles such as in the perinuclear spaces (PS) and rough endoplasmic reticulum (rER). In addition, vWF:Ag was identified in association with the large vesicles in the luminal cytoplasm. No reaction product was found in the cytosol. The basement membranes and the surrounding interstitium were barely labeled with vWF:Ag (Fig 4).

Thirty and 60 minutes after epinephrine administration, vWF:Ag could barely be observed in PS and rER. On the other hand, the luminal and basal plasma membranes, the basement membranes, and the surrounding interstitium were labeled with vWF:Ag. The cytoplasm contained abundant endocytic invaginations of the vWF:Ag-positive basal plasma membrane and vWF:Ag-containing vesicles, particu-

Fig 2. Immunohistochemical staining for vWF:Ag in sections from oral mucosa of DDAVP group. (a) Before administration, endothelial cells are strongly stained. (b) Thirty minutes and (c) 60 minutes after DDAVP administration (0.4 µg/kg). vWF:Ag-positive granules is sparse and coarse. Original magnification x 150.
Fig 3. Immunohistochemical staining for vWF:Ag in sections from oral mucosa of control group. (a) Before, (b) 30 minutes, and (c) 60 minutes after start of operation. No significant changes of staining are identified. (a) Original magnification ×210; (b) original magnification ×230; (c) original magnification ×245.

Fig 4. Immunoelectronmicroscopy before administration of DDAVP. vWF:Ag was localized in the protein-synthetic organelles of endothelial cells, such as perinuclear spaces (PS) and rER, and in association with large vesicles of luminal cytoplasm (arrows); bar = 1 μm.

Fig 5. Immunoelectronmicroscopy 60 minutes after administration of epinephrine (5.9 μg/kg). vWF:Ag can barely be observed in PS and rER of endothelial cells. On the other hand, basement membrane and surrounding interstitium are labeled for vWF:Ag. The dense precipitate is localized along the axis of collagen fibers (arrows); bar = 1 μm.

DISCUSSION

Localization of vWF:Ag in the endothelial cells of capillary in oral mucosa. Immunoelectronmicroscopically, vWF:Ag was demonstrated in rER, vesicles, and Golgi

larly along the basal surface. The reaction products were also observed in the lumen of capillaries (Fig 5). These findings were compatible with those after DDAVP administration (Fig 6).
Fig 6. Immunoelectronmicroscopy 60 minutes after administration of DDAVP (0.4 μg/kg). There is no significant difference between DDAVP and epinephrine. (a) Luminal and basal plasma membranes of endothelial cells. (a, b) the basement membranes and surrounding interstitium were labeled with vWF:Ag. Abundant endocytic invaginations of the vWF:Ag-positive plasma membrane are present (arrows); bar = 1 μm.

apparatus in endothelial cells of human vessels. Wagner and colleagues and Warhol and co-workers demonstrated that Weibel-Palade bodies, which were unique to endothelial cells, contained more intense vWF:Ag than did rER, vesicles, and vacuoles.

In the present study, we demonstrated vWF:Ag in rER, PS, and vesicles in the apical cytoplasm before administration of epinephrine or DDAVP, although Weibel-Palade bodies with vWF:Ag were not identified. In contrast to a previous study, localizations of this antigen in the basement membrane and the basal plasma membrane were not clear. This suggests that vWF:Ag might be released mainly to the capillary lumen, not to the subendothelium or interstitium, in the unstimulated state. These discrepancies appear to reflect differences in the nature of endothelial cells used, ie, capillaries in this study and arteries or veins in the previous study.

Kwast and associates, using a panel of monoclonal antibodies directed to factor VIII-vWF and a sensitive immunoperoxidase staining technique, showed that subendothelial deposition of vWF:Ag could be discerned only in arteries, arterioles, and large veins.

Localization change of vWF:Ag in endothelial cells by epinephrine or DDAVP. Because the rise of plasma VIII:C and vWF:Ag levels induced by epinephrine is inhibited by β-adrenoceptor blockade, an adrenergic mechanism appears to be involved in the release of vWF:Ag from vascular endothelium. The rise of factor VIII induced by DDAVP is independent of β-adrenoceptor, since propranolol has no influence on DDAVP’s effect on the release of factor VIII. DDAVP presumably sets free a humoral transmitter from brain. However, direct vascular action of DDAVP cannot be ruled out completely, because DDAVP directly increases platelet adhesion and spreading at injury sites when a perfusion model of illosogous human umbilical vein is used.

In the present study, vWF:Ag staining became fainter after epinephrine and DDAVP administration at the light microscopic level, and electronmicroscopically little vWF:Ag was identified in rER, PS, and cytoplasmic vesicles in which the antigen had been present. The mechanism for the absence of the antigen in rER has not yet been fully clarified. The localization of vWF:Ag changed to the basement membrane and the luminal surface of the plasma membrane. In addition, there were abundant endocytic invaginations of the vWF:Ag-positive basal plasma membrane and vWF:Ag-containing vesicle in the cytoplasm along the basal surface. There was no significant difference between the findings after epinephrine or DDAVP administration despite the different mechanisms. This suggests that epinephrine and DDAVP cause vWF:Ag secretion not only to the lumen of capillary but also to subendothelium by reverse pinocytosis from organelles responsible for protein synthesis, such as rER and PS. Rand and colleagues showed that vWF:Ag is present in cytoplasm of endothelial cell and in superficial proteins of the media associated with elastic lamina of adult human saphenous vein and splenic artery; they also suggested that subendothelial vWF:Ag might play a role in the adhesion of platelets to subendothelial components following endothelial injury. Our study showed that the hemostatic effect by local epinephrine injection probably depends not only on contraction of capillaries but also on secretion of vWF:Ag from endothelial cells to subendothelial areas and that this promotes the adhesion of platelets to basement membrane following endothelial injury during surgery. Similarly, the hemostatic effect of DDAVP in patients with vWD
may result not only from the rise of plasma VIII:C and vWF:Ag but also from secretion of vWF:Ag to subendothelial areas.

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