

# EFFECT OF VASOACTIVE AMINES AND RESERPIN ON THE WEIBEL-PALADE BODY CONTENT IN GUT MUCOSAL VESSELS: AN ULTRASTRUCTURAL AND MORPHOMETRIC STUDY

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**Keywords:** gut mucosal capillaries — endothelial cell — Weibel-Palade body — morphometry

Endothelium-specific Weibel-Palade bodies (WPb) possess a dense matrix- and tubular structures which most probably present the morphologic image of von Willebrand proteins in a condensed form (6). Von Willebrand factor (vWF) is a high-molecular adhesive glycoprotein mediating platelet adhesion to basement membrane and participating in thrombocyte aggregation (75). According to various investigations (7), its biosynthesis is performed in the rough endoplasmic reticulum and in the Golgi complex after which a great part of synthesized product is concentrated in WPb. Its secretion is stimulated by thrombin, calcium ionophore A23187 and other substances. vWF level increase results from various physiological and pharmacological influences (2, 3) and can be observed in some pathological conditions and diseases such as glaucoma and renal hypertension. Therefore, besides its synthesis many problems concerning vWF secretion rate and stimulation have to be solved.

The aim of the present study is to evaluate the influence of some vasoactive amines and their depletor reserpin in vivo on WPb content and distribution in gut mucosal vessels.

## Material and methods

Serotonin creatinine-phosphate (Sigma) in a dose of 150 µg/100 g b. w. was intraperitoneally injected to 6 normotensive Wistar male rats. Material was taken from stomach and large-intestine mucosa on the 15th and 60th min after injection. Four normotensive rats were infused via the left ventricle with 100 µg/100 g b. w. serotonin for 60 sec. Material was taken immediately after infusion. Histamine hydrochloride (Buchs, Switzerland) was intravenously applied into the femoral vein in a dose of 125 and of 250 µg/100 g b. w. to 12 normotensive animals. Material was taken on the 90th and 210th sec after injection. Reserpin was administered intraperitoneally in a dose of 2.5 mg/kg b. w. to two rats in order to stimulate endogenous serotonin deposits. Material was taken after 19 hours. This time interval is considered optimal concerning reserpin action. Controls were treated with the same volumes of vasoactive drug solvents. Samples were prepared by means of standard procedure for transmission electron microscopy. Observation was made by JEM 7A and Opton 109 EM-Turbo electron microscopes. WPb number per µm<sup>2</sup> was calculated on electronograms of capillary profiles perpendicular to vascular section with control ani-

mals, after serotonin infusion and after intraperitoneal reserpin administration. As the two latter trials demonstrated the most significant changes of WPb content in capillary endothelial cells a comparative morphometric investigation of them was carried out. Data obtained were presented on histograms and statistically processed by the variance analysis.

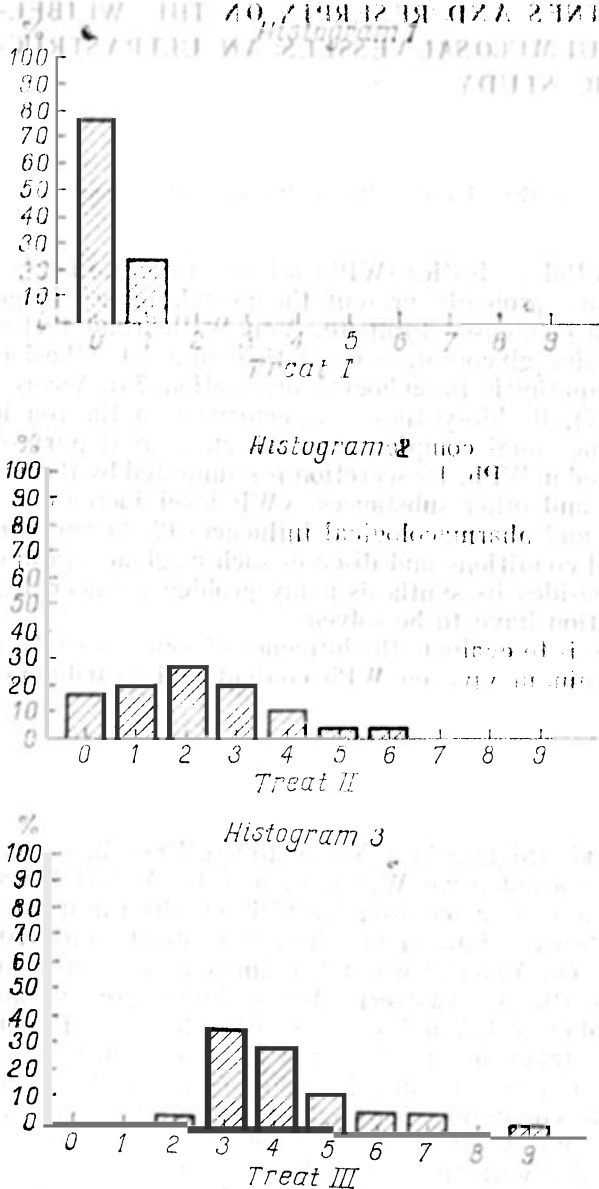


Fig. 1. WPb distribution in controls (treat I), after serotonin infusion (treat II) and reserpin administration (treat III). Histograms shifted to the right in experimental animals. X-axis indicated WPb density and Y-axis — percentage of cases.

## Results and discussion

WPb in endothelial cells of terminal mucosal blood vessels are a very rare finding. They occur as single bodies in the periphery of the endothelial cell. This relates mainly to arterioles and venules while WPb presence in capillaries is an extraordinarily rare phenomenon. One WPb is observed only in 23 per cent of sections examined each (fig. 1), and WPb density amounts to  $0.30 \text{ WPb}/\mu\text{m}^2$  (table 1). However, 15 min after intraperitoneal serotonin injection, their number increases with all microvessels. In some capillaries they appear in twos and threes in their usual localization. 60 min after 5-HT administration, endothelial cells do not demonstrate significant changes as compared with these in control animals. WPb count increases significantly after serotonin infusion. One up to 6 WPb are observed in 83 per cent of capillary cuts (fig. 1) and their density reaches up to  $2.52 \text{ WPb}/\text{m}^2$  (table 1). WPb occur often in the so-called «secretory position» — fused with the luminal endothelial cytomembrane. Reserpin induces the most significant WCb count increase; at the average up to  $4.65 \text{ WPb}/\text{m}^2$  (table 1). WPb are established in all the sections examined. Their number varies between 2 and 9 but most frequently they are 3 or 4 in section each

Table 1  
WPb number in one EC of capillaries studied

	control	serotonin inf. 100 µg/100 g	reserpin intrapérit.
WPb/m <sup>2</sup>	0.30	2.52	4.65
standard deviation	1.49	1.56	2.36
capillary number	30	30	30

(fig. 1). Histamine in a dose of 250 µg/100 g b. w. for 90 sec induces a considerable WPb number increase, mainly in gastric mucosa arterioles and large-intestinal mucosa capillaries. WPb reach up to 20 in number per endothelial cell with gastric arterioles (the normal value is between 2 and 5). They are localized in any parts of the endothelial cell even in the immediate proximity of the interendothelial contact which is a very rare phenomenon under normal conditions. WPb accumulation is combined by dilatated cisternae of endoplasmic reticulum.

Our results indicate that WPb count in microcirculatory vessels increases after treatment with vasoactive monoamines and reserpin. This effect is more manifested after intravasal amine application and to a greatest extent after reserpin administration. Their increase is more evident with capillaries most probably because of the extraordinary rarity of WPb presence in normal capillary endothelial cells. It can be supposed that WPb number increase could induce a greater probability for microvascular coagulation. Other vasoactive substances such as vasopressin and adrenalin have stimulated vWF secretion (1). However, endothelial tissue cultures treated with serotonin failed to increase vWF level in the medium (6). In this case, the authors assume that in vitro model does not suit all the requirements for manifestation of endothelial haemostatic properties. Our data obtained do not enable the evaluation if the increased number of these organelles means an increased vWF synthesis or secretion or monoamines only stimulate adhesive glycoprotein condensation into WPb. Despite all we presume that WPb count elevation influenced upon by monoamines implies an increase of the quantity of vWF-multimers or high biological activity and better expressed potential adhesive capacity of endothelial cells.

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**ЭФФЕКТ ВАЗОАКТИВНЫХ АМИНОВ И РЕЗЕРПИНА НА НАЛИЧИЕ ТЕЛЕЦ ВЕЙБЕЛЬ-ПАЛАДЕ В СОСУДАХ СЛИЗИСТОЙ ОБОЛОЧКИ ПИЩЕВАРИТЕЛЬНОГО ТРАКТА: УЛЬТРАСТРУКТУРНОЕ И МОРФОМЕТРИЧЕСКОЕ ИССЛЕДОВАНИЕ**

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**РЕЗЮМЕ**

Методом трансмиссионной электронной микроскопии исследовано влияние гистамина, серотонина и резерпина на количество и распределение телец Вейбеля-Паладе в эндотелиальных клетках терминальных кровеностных сосудов слизистой оболочки пищеварительного тракта крыс. Морфометрические данные статистически обработаны и представлены на гистограммах. Устанавливается, что вазоактивные амины и резерпин приводят к повышению эндотелиальных клеток всех терминальных сосудов слизистой оболочки, но количество этих клеток относительно наиболее высоко в эндотелиальных клетках капилляров после интраартериальной инфузии серотонина и после введения резерпина. По сравнению с контрольными животными, у которых тельца Вейбеля-Паладе являются очень редкой находкой (в среднем 0,30р м<sup>2</sup>), после воздействия серотином их количество нарастает до 2,52р м<sup>2</sup>, а после применения резерпина их среднее число увеличивается до 4,65р м<sup>2</sup>. Тельца Вейбеля-Паладе содержат адгезивный гликопротеин — фактор фон Вилебранд, который медирует тромбоцитную адгезию к базальной мембране сосудов. В результате воздействия вазоактивными моноаминами количество телец Вейбеля-Паладе повышается за относительно короткий период времени. Это явление можно квалифицировать как морфологическую готовность к повышенной прокоагуляционной активности в микрососудах.

Our results indicate that WFP count in endothelial cells after treatment with vasoactive amines and reserpine is maintained after intravascular amine application and is a greater effect from reserpine administration. Their increase is more evident with capillary endothelium. This increase of WFP probably because of the extraordinary large of WFP number in capillary endothelial cells. It can be supposed that WFP number increase indicates a greater probability for microvascular thrombocytopenia. The increase of endothelial cells with WFP is probably due to the fact that the number of WFP in the endothelial cells is not only a function of the number of endothelial cells but also of the number of WFP in each cell. The number of WFP in each cell is not only a function of the number of endothelial cells but also of the number of WFP in each cell. The number of WFP in each cell is not only a function of the number of endothelial cells but also of the number of WFP in each cell.

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