Z Rechtsmed (1989) 103:85–91

Zeitschrift für
Rechtsmedizin

Original works

Histamine effects on pulmonary blood vessels in strangulation

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Summary. Endothelial cells of asphyxial pulmonary veins possess abundant pores and intracytoplasmic vacuoles. The present radioassay demonstrated an increase in histamine concentrations of the pulmonary tissue in asphyxia. These morphological changes, therefore, appear to represent an enhancement of the endothelial permeability induced by high histamine concentrations in blood plasma. The present immunoelectron microscopy study demonstrated heavy reactions of histamine exclusively on the endothelial surface of the asphyxial pulmonary veins. This may support the endothelial cell-dependent vasodilation mediated by histamine in asphyxial pulmonary veins.

Key words: Asphyxia, immunocytochemistry – Strangulation, endothelial cell-dependent vasodilation

Zusammenfassung. Die Endothelzellen der asphyxialen Lungenvenen besitzen eine überreiche Anzahl an Poren und intracytoplasmischen Vakuolen. Da bei Erstickung der Anstieg an Histamin-Konzentration des Lungengewebes mittels Messung der Radioaktivität festgelegt wird, scheinen diese morphologischen Veränderungen in dem durch hohe Histamin-Konzentration verursachten Anstieg an endothelischer Durchlässigkeit wiedergegeben zu sein. Die vorliegende Immunoelektro-Mikroskopie beschreibt die starken Histamin Reaktionen ausschließlich auf der endothelischen Oberfläche der asphyxialen Lungenvenen. Die Ergebnisse unterstreichen die Annahme, daß die Endothelzellen bedingte Vasodilatation mittelbar auf das Histamin in den asphyxialen Lungenvenen zurückgeführt werden kann.

Schlüsselwörter: Erstickung, Immuno-Cytochemie – Strangulierung, Endothelzellen-bedingte Vasodilatation

Introduction

In various pharmacological actions of histamine on pulmonary vessels, McNamee (1983) observed an increase in the vascular permeability, and Satoh

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and Inui (1984) considered the possibility of endothelial cell-dependent vasodilation by histamine. According to Douglas (1985), the vasodilation and increase of vascular permeability that occur in the blood vessels appear to be mediated by histamine acting predominantly through H_1-receptors. On the other hand, an increase in concentration of plasma histamine has been reported in asphyxia (Prokop and Göhler 1976), and our previous high pressure liquid chromatography (HPLC) study indicated high levels in concentration of plasma histamine in asphyxiated guinea pigs (Kita et al. 1987). However, we have little morphological data regarding the effects of such an increased plasma histamine on the pulmonary circulation in asphyxia, although our preliminary observations demonstrated the constriction of the pulmonary arteries and the capillaries and the dilation of the veins in these animals (Kita et al. 1986). More detailed morphological studies including immunocytochemistry are necessary to elucidate the effects of histamine on vasomotion.

On these grounds, the present immunoelectron microscopy study was done to observe the localization of histamine in the pulmonary vessels in asphyxiated guinea pigs. In addition, the difference in histamine concentration of the pulmonary tissue between asphyxial and control groups was determined by radioassay.

Materials and Methods

Male Hartley guinea pigs weighing 480–550 g were used for the present study. Animals for the asphyxial group were strangled using gauze, and those for the control group were killed by cutting the carotid artery; control animals for radioassay were killed by striking the occiput. The animals used in these experiments were killed after anesthetization with ether.

For scanning electron microscopy (SEM), the pulmonary vessels were infused with a solution of 0.1 *M* sodium cacodylate buffer and then with a Karnovsky solution at 4°C (Karnovsky 1965) from the inferior vena cava. The lungs were isolated, cut into 2-mm thick slices, and postfixed in 2% osmium tetroxide in 0.1 *M* phosphate buffer at 4°C for 1 h. Specimens were dehydrated in ascending alcohol series, dried using the critical point method, coated with platinum-palladium, and examined with a Hitachi S-700 type field emission SEM. The streptavidin-biotin technique was used for the present immunoelectron microscopy. After the infusion of 1% sodium metabisulfite in 0.1 *M* sodium cacodylate buffer via the inferior vena cava, the pulmonary vessels were rapidly perfused with a solution of 5% glutaraldehyde and 1% sodium metabisulfite in 0.1 *M* cacodylate buffer. The lungs were cut into 2-mm thick slices and refixed in the same fixation for 1 h. Sections 40 μm thick were made using a microslicer (Dosaka EM) and inserted into the sample meshpack (Shiraimatsu). After treatment cubated with polyclonal antihistamine antibody (Chemicon International) at 4°C overnight. The working dilution of the antiserum was 1:200 in 0.05 *M* TRIS buffer containing 0.85% sodium metabisulfite. After incubation, the sections were reacted with biotinylated goat antirabbit immunoglobulins (Stravigen Immunostaining Kits, BioGenex Laboratories, Dublin, Calif.), and then with 0.05% 3,3′-diaminobenzidine tetrahydrochloride (Sigma) in 0.05 *M* TRIS buffer containing H_2O_2 (pH 7.6) at room temperature for 10 min. At every step of the incubations, the sections were washed in 0.05 *M* TRIS buffer. They were postfixed in 1% osmium tetroxide in 0.1 *M* phosphate buffer at 4°C for 1 h, dehydrated in ascending alcohol series, embedded in Quetol 812, and observed with a JEM 100CX electron microscope. In order to confirm the specificity of the immunocytochemical labelings, the following controls were performed: (1) the antihistamine serum was absorbed with an excess histamine and (2) either normal guinea-pig serum or TRIS buffer was substituted for the goat antirabbit serum.

For radioassay, both strangulated and control animals were injected with 10 μCi ^{3}H-histamine (specific activity was 32.1 Ci/mmol, Dupont) via the inferior vena cava immediately after respiratory arrest. After cardiac arrest, the lungs were rapidly perfused with 0.1 *M* sodium cacodylate buffer and then with 2.5% glutaraldehyde in 0.1 *M* sodium cacodylate buffer from inferior vena cava. The isolated lungs were cut into 3-mm-thick slices. Six slices randomly chosen were treated with an automatic sample combustion system (Aloka ASC-113) and counted using a liquid scintillation system (Packard TRI-CARB 2600).

Results

SEM pictures demonstrated abundant pores of varying size exclusively in the endothelia of the pulmonary veins of the asphyxial group (Fig. 1).

Such endothelial pores appear to be more numerous at the periphery of the cells. The endothelial surface of control animals remains intact as shown in Fig. 2.

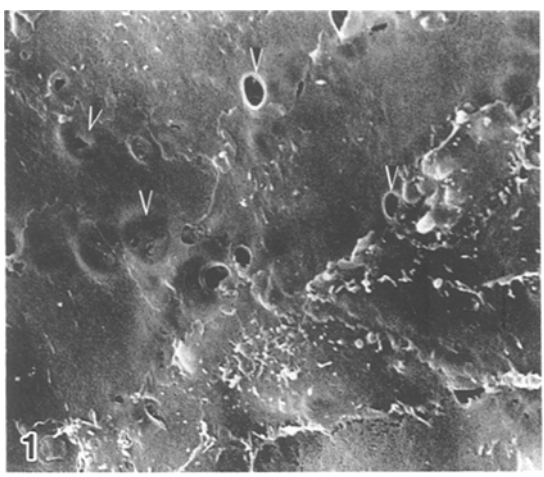

Fig. 1. Scanning electron micrograph of an asphyxial pulmonary vein shows the existence of abundant pores *(arrowheads)* of the endothelial cells; × 3,100

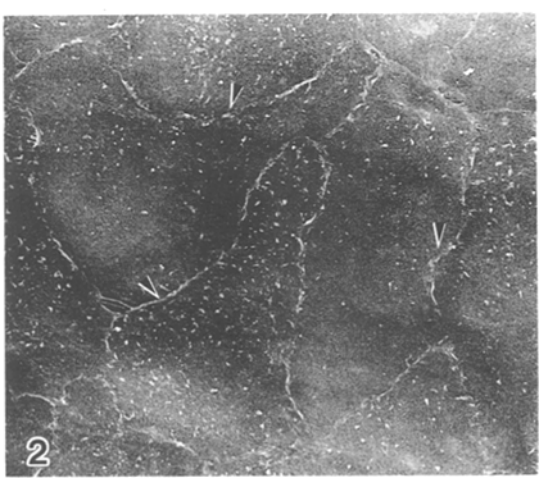

Fig. 2. A pulmonary vein in bleeding to death does not possess endothelial pores as shown in Fig. 1. The endothelial boundary is well preserved *(arrowheads);* × 1,600

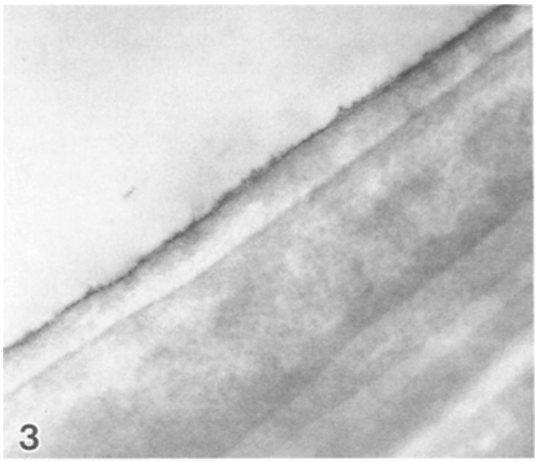

Fig. 3. Positive immunoreactions of histamine are located on the endothelial surface of an asphyxial pulmonary vein; × 31,000

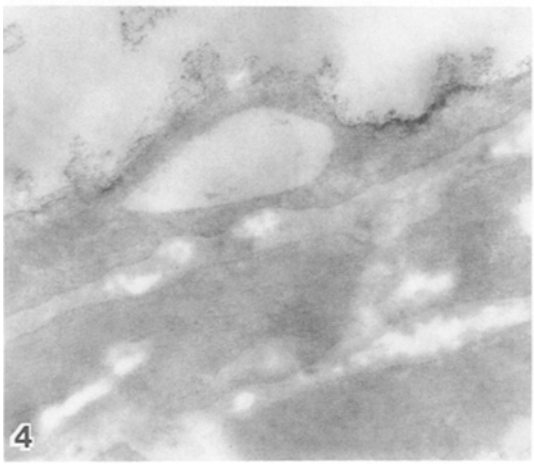

Fig. 4. The asphyxial pulmonary vein possesses an intraendothelial vacuole. Immunoreactions of histamine are also positive on the luminal endothelial surface; × 25,000

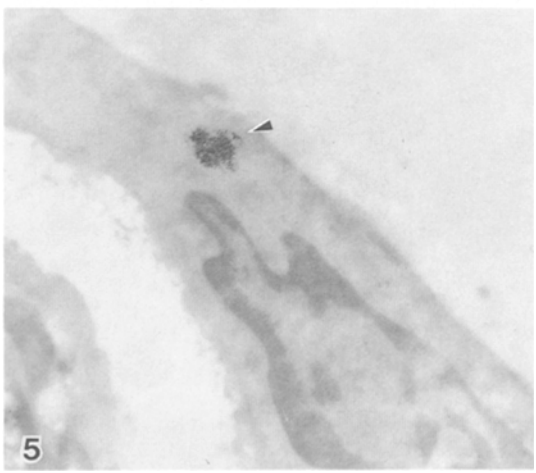

Fig. 5. No immunoreactions of histamine are located on the endothelial surface of the asphyxial pulmonary artery, while a Weibel-Palade body *(arrowhead)* shows the positive reactions; × 19,000

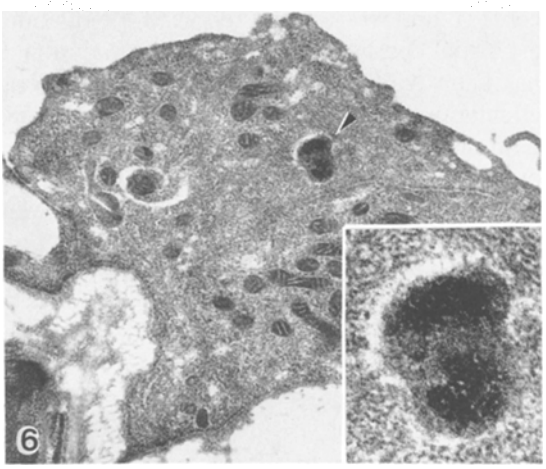

Fig. 6. Positive immunoreactions of histamine in a Weibel-Palade body of the pulmonary artery are shown, stained with lead citrate; × 15,000. *Inset* shows the highly magnified Weibel-Palade body; × 62,500

Table 1. ^{3}H-Histamine contents (μCi/g) of the pulmonary tissues following i.v. administration at a point of time immediately after respiratory arrest. (Mean values of six sections ± SD)

Means of killing	Guinea pig no.			Mean ± SD
	1	2	3	
Strangulation	0.239 ± 0.029	0.213 ± 0.043	0.179 ± 0.020	0.210 ± 0.030
Cutting of carotid	0.152 ± 0.017	0.160 ± 0.011	0.110 ± 0.010	0.141 ± 0.027*
Striking on occiput	0.156 ± 0.021	0.155 ± 0.022	0.169 ± 0.023	0.160 ± 0.008*

* Significantly different from strangulation, $P < 0.05$

Immunoreactions to histamine were preferentially localized on the luminal surface of the venous endothelia of the asphyxial group (Fig. 3).

In addition, we often encountered abundant intracytoplasmic vacuoles exclusively in the endothelial cells of this group as shown in Fig. 4.

The immunoreactions on the endothelial surface of the pulmonary arteries of both asphyxial (Fig. 5) and control groups were not apparent.

Another interesting finding was that Weibel-Palade bodies of the arterial endothelia in the asphyxial group showed heavy immunoreactions to histamine (Figs. 5, 6).

Neither pulmonary arteries of the bleeding group nor pulmonary veins of both groups revealed any specific reactions of histamine on these granules.

In the present radioassay, a remarkable increase in concentration of ^{3}H-histamine in the pulmonary tissues was evident in the asphyxial group compared with the controls (see Table 1, $P < 0.05$). There were no significant differences in histamine contents between the two kinds of controls.

Discussion

Satoh and Inui (1984) described the endothelial cell-dependent vasodilation of the guinea-pig pulmonary artery as being possibly mediated by histamine H_1-re-

ceptors, and we recently reported the dilatation of the pulmonary veins in the asphyxial condition with an image analysing device (Kita et al. 1986). Since it has been confirmed that histamine concentrations increase in the pulmonary circulation in asphyxiated animals, a role of histamine in the vasomotion of the pulmonary vessels might be worthy of note. The present immunoelectron microscopy indicated that positive histamine reactions were located exclusively on the luminal surface of the pulmonary venous endothelia. Therefore, it is reasonable to suppose that the vasodilation of the pulmonary veins induced by high histamine concentrations in plasma is mediated by H_1-receptors on the endothelial surface. This speculation argues for our data that endothelial cells of the pulmonary arteries do not show immunoreactions to histamine and that the pulmonary arteries do not dilate by an elevation of plasma histamine concentrations in asphyxia.

McNamee (1983) considered that histamine was able to increase the permeability of the pulmonary vessels, and Meyrick and Brigham (1984) claimed that the increase in endothelial permeability of the bovine pulmonary arteries after application of histamine was mainly due to transient dissociations of the interendothelial junctions. Although the present observations did not show such morphological signs in the asphyxial pulmonary arteries, abundant pores and intracytoplasmic vacuoles were observed in the endothelia of the asphyxial pulmonary veins. These morphological alterations may imply that an increase in endothelial permeability of the pulmonary veins is induced by an increase in plasma histamine concentrations in asphyxia. However, whether histamine receptors of the venous endothelia are involved in such an enhanced permeability remains uncertain.

Why the pulmonary arteries become constricting in proportion to an increase in plasma histamine concentrations in asphyxia is difficult to explain from the present data. For solving this problem, a role of Weibel-Palade bodies in the pulmonary vasomotion should be considered since our immunoelectron micrographs revealed positive reactions of histamine on these granules in the asphyxial arteries. Hauge (1968) suggested the involvement of endogenous histamine in the vasoconstriction in acute alveolar hypoxia, and Reid and Meyrick (1980) showed an increase in number of Weibel-Palade bodies in the hypoxial pulmonary arteries. Taking these data into consideration, more detailed studies, including immunoelectron microscopy on histamine receptor sites in the pulmonary vessels, are now in progress in our laboratory to elucidate how histamine taken up into endothelial cells from plasma mediates vasodilation in the asphyxial pulmonary veins and whether endogenous histamine released from Weibel-Palade bodies is involved in the vasoconstriction in the asphyxial pulmonary arteries.

Acknowledgements. We wish to thank Prof. S. Fujimoto, Department of Anatomy, for critically reading this manuscript and Miss M. Minoda for her skillful technical assistance. This work was supported in part by a grant-in-aid for scientific research (no. 63570280) from the Ministry of Education in Japan.

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Received December 12, 1988