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Catecholamine Effects on Pulmonary Blood Vessels in Strangulation

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Summary. Guinea pigs were killed by strangulation to investigate the vasomotor of pulmonary congestion by asphyxia. The noradrenaline uptake by the endothelial cells of the pulmonary arteries and capillaries was observed by fluorescence histochemistry, peroxidase-anti-peroxidase (PAP) immunocytochemistry, and autoradiography. By radioassay, the volumes of noradrenaline uptake by the pulmonary arteries and capillaries in the strangulation group were at a significantly higher level than in the control groups. Many myoendothelial junctions were observed at the sites of constricted arteries, and the invasive noradrenaline was clearly observed in the myoendothelial junction.

The mechanism of the pulmonary vasoconstriction in strangulation is due to the fact that the plasma noradrenaline, increased by asphyxia, invades the endothelial cells of pulmonary arteries and capillaries and causes the vasoconstriction.

Key words: Asphyxia, vasoconstriction of pulmonary arteries – Strangulation, noradrenaline

Zusammenfassung. Erdrosselte Meerschweinchen wurden auf die Vasomotorik der Lungenstauung bei Erstickungstod untersucht. Die Noradrenalinaufnahme in die Endothelzellen der Pulmonalarterien und Kapillaren wurde durch Fluoreszenz-Histochemie, Peroxidase-Antiperoxidase (PAP), Immuno-Cytochemie und Autoradiographie gemessen. Im Radio-Assay zeigte die erdrosselte Gruppe einen signifikanten Anstieg der Noradrenalin-Aufnahmemenge in die Lungenarterien im Vergleich zur Kontrollgruppe. Viele myoendotheliale Verbindungspunkte wurden an den Stellen der kontrahierten Arterien beobachtet, und das eingedrungene Noradrenalin wurde in den myoendothelialen Verbindungspunkten nachgewiesen.

Der Mechanismus der Lungen-Vasokonstriktion bei Erdrosselung ist dadurch bedingt, daß bei Erstickung das Plasma-Noradrenalin ansteigt, in die endothelialen Zellen der Lungenarterien und -Kapillaren eindringt und schließlich die Vasokonstriktion verursacht. Auf eine weitergehende Diskussion bezüglich der Übertragbarkeit der Untersuchungsergebnisse auf die Verhältnisse beim Menschen wird zunächst verzichtet.

Schlüsselwörter: Erstickung, Vasokonstriktion der Lungenarterien – Strangulation, Noradrenalinanstieg

Introduction

It is well known that an increase in plasma catecholamines and pulmonary congestion is observed in asphyxia (Prokop and Göhler 1976). There have been a number of studies concerning the uptake of amines by the lung. Noradrenalin (Ginn and Vane 1968; Hughes and Gillis 1968; Hughes et al. 1969; Iwasawa et al. 1973) and serotonin (Al-Ubaidi and Bakhle 1980; Gillis et al. 1979; Iwasawa et al. 1973; Kjellström et al. 1984; Strum and Junod 1972) are partially or completely removed from the blood during these passages through the lung, whereas adrenaline (Boileau et al. 1971; Ginn and Vane 1968) and dopamine (Boileau et al. 1972) are not removed. The effect of the increased plasma catecholamine by asphyxia on the pulmonary blood vessels has not yet been reported. The author has studied the mechanism of the pulmonary congestion in strangulation. To achieve this, the ratios of constrictions of the pulmonary arteries were calculated by a scanning electron microscope, and the minimum diameters of the alveolar capillaries and the pulmonary veins were measured by light microscope. It has been observed that the pulmonary arteries and capillaries were constricted and the pulmonary veins dilated (Kita et al. 1986a). The changes of levels of plasma vasoconstrictive substances (adrenaline, noradrenaline, and serotonin) were studied by a high pressure liquid chromatography, and it was observed that the level of plasma noradrenaline rapidly decreased and adrenaline rapidly increased during the intervals from the respiratory arrest to the cardiac arrest (Kita et al. 1986b).

The author observed the localization of catecholamines in the pulmonary blood vessels by fluorescence histochemistry, PAP immunocytochemistry to anti-noradrenaline antibodies, and autoradiography. Moreover, the differences in the levels of catecholamine uptake between the experimental group and control groups were investigated by radioassay. In this report, the relation between the sites of catecholamine in the lung and the vasoconstrictions caused by asphyxia were studied.

Materials and methods

Animals

Fifty male Hartley guinea pigs (480–550 g b. wt.) were used.

Means of Killing

Animals in the experimental group were strangled. In the control group, animals were killed by bloodletting (cutting of the carotid), and furthermore in the control group at the experiment of the radioassay, animals were killed by striking on the occiput.

Fluorescence Histochemistry

Specimens were prepared according to the method of Falck et al. (1962) and Furness et al. (1977). Furthermore, the pulmonary tissues were fixed with 4% paraformaldehye and 0.5% glutaraldehyde, and the exposal interval to formaldehyde gas at 80°C was for 1 h. The specific amine fluorescence reaction was tested by the borohydride reduction test (Corrodi et al. 1964). An inverted fluorescence microscope was used (Carl Zeiss ICM 405) and the following filter combination was employed: exciting filter BP 400–440 nm, dichroic mirror FT 460 nm, barrier filter LP 470 nm.

Electron Microscope Immunocytochemistry

The author applied the unlabeled PAP immunocytochemistry technique to anti-noradrenalin antibodies at ultrastructural levels. After the infusion of 1% sodium metabisulfite in 0.1 *M* sodium cacodylate buffer into the pulmonary blood vessels from the vena cava inferior, the pulmonary tissues were rapidly perfused with an ice-cold solution containing 5% glutaraldehyde and 1% sodium metabisulfite in a 0.1 *M* sodium cacodylate buffer. Removed lungs were cut into 2 mm thick slabs and postfixed for 1 h in the same fixative. Forty-micrometer-thick sections were cut on a microslicer (Dosaka EM CO., Ltd.), and these sections were packed into the sample-mesh-pack (Shiraimatsu & Co., Ltd.), and processed for immunocytochemistry on the basis of the PAP technique of Onteniente et al. (1984). After the immunocytochemistry procedure, the sections were taken out of the sample-mesh-pack and flat-embedded in Quetol 812. Ultrathin sections were examined with a JEM 100CX electron microscope without further staining.

Radioassay

Animals were injected i.v. with 10 μCi of the ^{3}H-noradrenaline (specific activity 15 Ci/mmol via the vena cava inferior, Amersham International plc) or ^{3}H-adrenaline (specific activity 7.51 Ci/mmol, Du Pont Co.) immediately after respiratory arrest. After the cardiac arrest, the pulmonary tissues were rapidly perfused through the vena cava inferior with 0.1 *M* sodium cacodylate buffer and were perfused with 2.5% glutaraldehyde. The removed lungs were cut into 3-mm-thick slabs, and six sections (random sampling) were treated with an automatic sample combustion system (Aloka ASC-113) and counted by a liquid scintillation system (Packard TRI-CARB 2660).

Autoradiography

Animals were injected i.v. with 500 μCi of the ^{3}H-noradrenaline (specific activity 15 Ci/mmol via the vena cava inferior, Amersham International plc) or ^{3}H-adrenaline (specific activity 7.51 Ci/mmol, Du Pont Co.) immediately after respiratory arrest. After the cardiac arrest, the pulmonary tissues were perfused via the vena cava inferior with 0.1 M sodium cacodylate buffer and were perfused with 2.5% glutaraldehyde. The removed lungs were cut into 1-mm-thick slabs and fixed with 2% osmium tetroxide. They were dehydrated in an ascending alcohol series and embedded in Quetol 812. Ultrathin sections were mounted on collodion-coated grids, stained with uranyl acetate and lead citrate, vacuumcoated with approximately 5-nm-thick carbon, and then coated with a monolayer of Sakura NR-H2 emulsion by a dipping method (Joftes and Warren 1955). After exposure in dark boxes containing silica gel at 4°C for 40 days, the sections on the grids were developed with Dektol (Kodak) at 20°C for

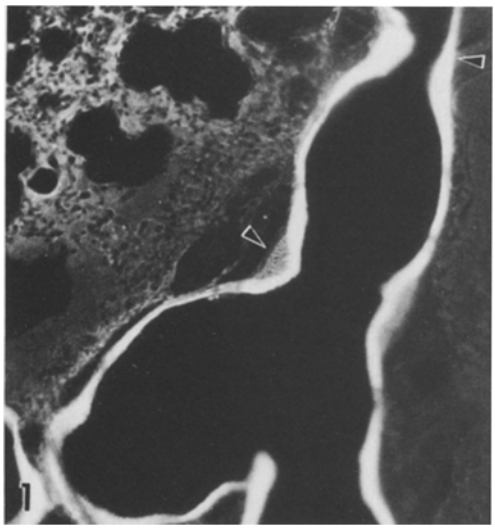


Fig. 1. Fluorescence micrograph of constricted pulmonary artery in strangulation. Specific bright green fluorescence of muscular artery endothelial cells. *Arrows* indicate the site of vasoconstricted parts. × 180

Fig. 2. Fluorescence micrograph of pulmonary artery in bleeding to death. Specific bright green fluorescence of muscular artery endothelial cells. × 400

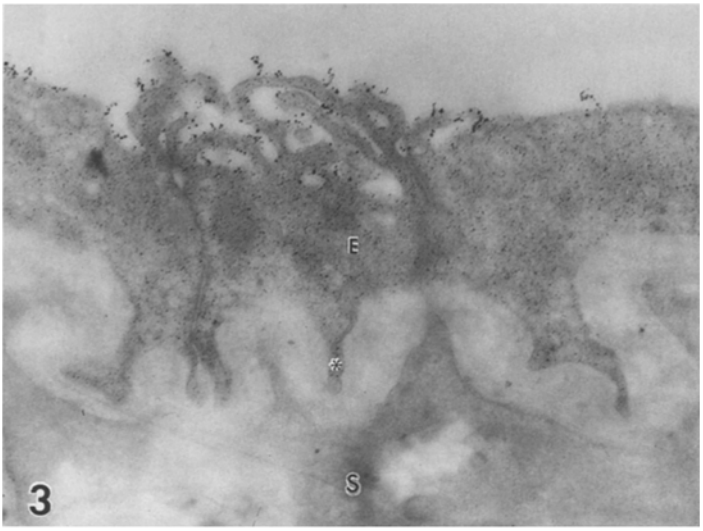
3 min and fixed in acid hypofixer for 3 min. After stripping the extra emulsion from the section with 0.1 *N* NaOH for 30 min, they were examined with a JEM 100CX electron microscope.

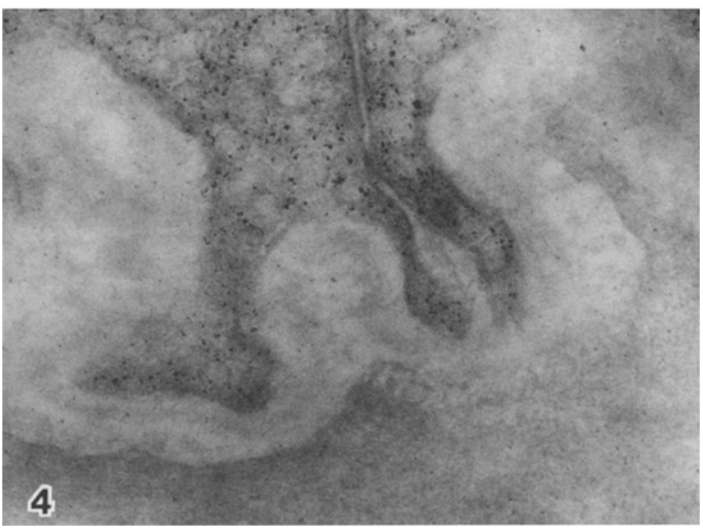
Results

Fluorescence Histochemistry

Specific bright green fluorescences were observed at the endothelial cells of the pulmonary arteries and arterioles. This fluorescence was observed in both the experimental and the control groups (Figs. 1, 2).

It was not clearly observed in the capillary endothelial cells, and was not observed in the vein endothelial cells.





Figs. 3, 4. Electron micrograph of constricted pulmonary artery endothelial cells *(E)* labeled by PAP immunocytochemistry with anti-noradrenaline antibodies in strangulation. The many myoendothelial junctions (*) at the sites of vasoconstricted arteries are observed (Fig. 3), and immunoreaction is clearly observed in the myoendothelial junction (Fig. 4). *S* Smooth muscle cell. Fig. 3 × 17,000; Fig. 4 × 45,000

Electron Microscope Immunocytochemistry

Noradrenaline invasion into pulmonary artery endothelial cells was observed in the experimental and control groups (Figs. 3, 5). Many myoendothelial junctions were observed at the site of constricted arteries, and noradrenaline invasion was clearly observed at the myoendothelial junction (Fig. 4). The invasion of noradrenaline was also observed in the capillary endothelial cells (Fig. 6), but not at the vein endothelial cells.

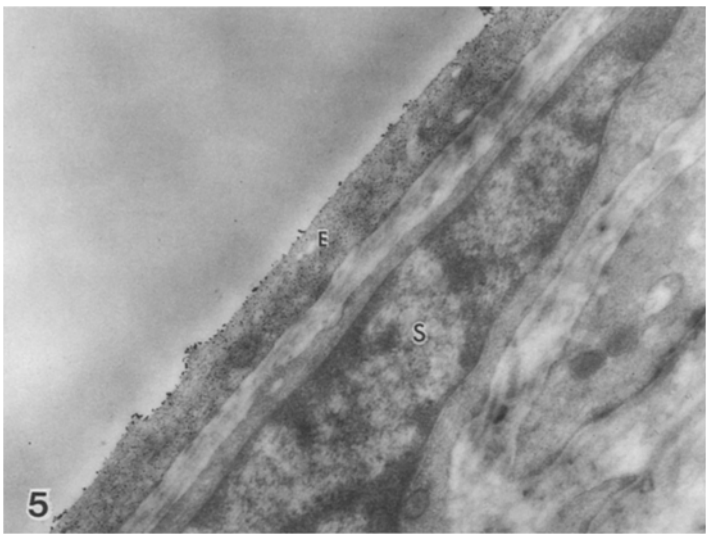


Fig. 5. Electron micrograph of pulmonary artery in bleeding to death. Pulmonary artery endothelial cell *(E)* labeled by PAP immunocytochemistry with anti-noradrenaline antibodies. *S* Smooth muscle cell. ×14,000

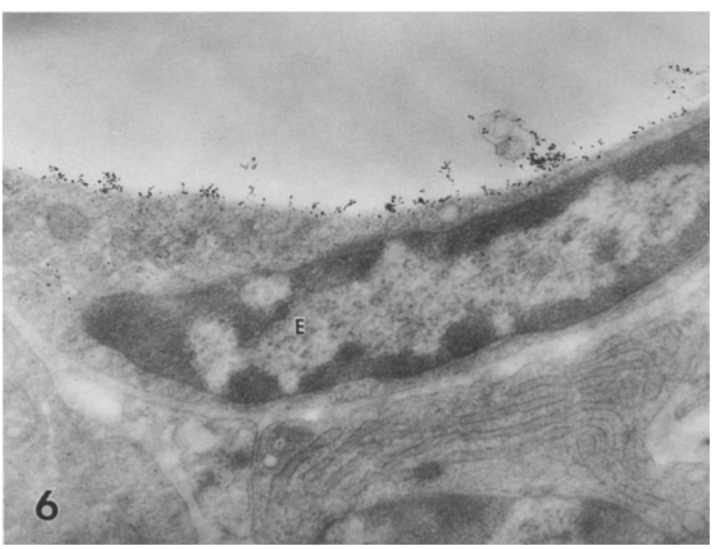


Fig. 6. Electron micrograph of pulmonary capillary in strangulation. Pulmonary capillary endothelial cell *(E)* labeled by PAP immunocytochemistry with anti-noradrenaline antibodies. ×20,000

Radioassay

In the experimental group, a significant difference was seen at the level of ^{3}H-noradrenaline in the pulmonary tissues as compared to the control groups (Table 1, $P < 0.01$). No significant differences were seen among the control groups.

Table 1. ^{3}H-noradrenaline contents of the pulmonary tissues following i.v. administration (μCi/g)

Means of killing	No. 1	No. 2	No. 3	Mean ± SD
Strangulation	0.364 ± 0.047[a]	0.318 ± 0.048	0.306 ± 0.007	0.329 ± 0.031
Cutting of carotid	0.128 ± 0.036	0.055 ± 0.034	0.126 ± 0.048	0.103 ± 0.042*
Striking on occiput	0.095 ± 0.035	0.112 ± 0.046	0.089 ± 0.031	0.099 ± 0.012*

[a] Mean values in six sections ± SD
Significantly different from the strangulation: * $P < 0.01$

Table 2. ^{3}H-adrenaline contents of the pulmonary tissues following i.v. administration (μCi/g)

Means of killing	No. 1	No. 2	No. 3	Mean ± SD
Strangulation	0.036 ± 0.014[a]	0.051 ± 0.011	0.032 ± 0.010	0.040 ± 0.010
Cutting of carotid	0.033 ± 0.009	0.048 ± 0.033	0.027 ± 0.024	0.036 ± 0.011
Striking on occiput	0.022 ± 0.002	0.024 ± 0.003	0.034 ± 0.006	0.027 ± 0.006

[a] Mean values in six sections ± SD

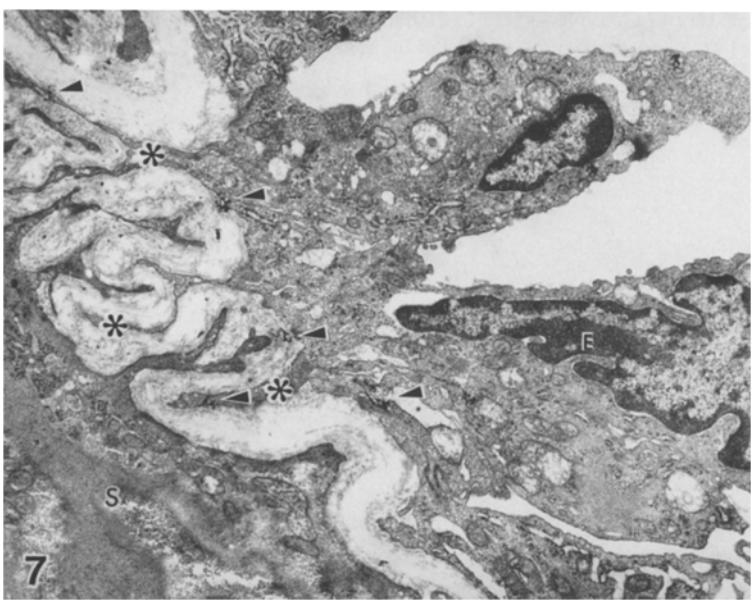


Fig. 7. Autoradiograph of constricted pulmonary artery in strangulation after ^{3}H-noradrenaline administration. Silver grains *(arrows)* are observed in the pulmonary artery endothelial cells *(E)* and myoendothelial junctions (*). *S* Smooth muscle cell. × 6,000

In the experimental and control groups, no significant differences were seen in the level of ^{3}H-adrenaline in the pulmonary tissues (Table 2).

Autoradiography

In the experimental and control groups after ^{3}H-noradrenaline administration some silver grains were observed in the pulmonary artery and capillary endo-

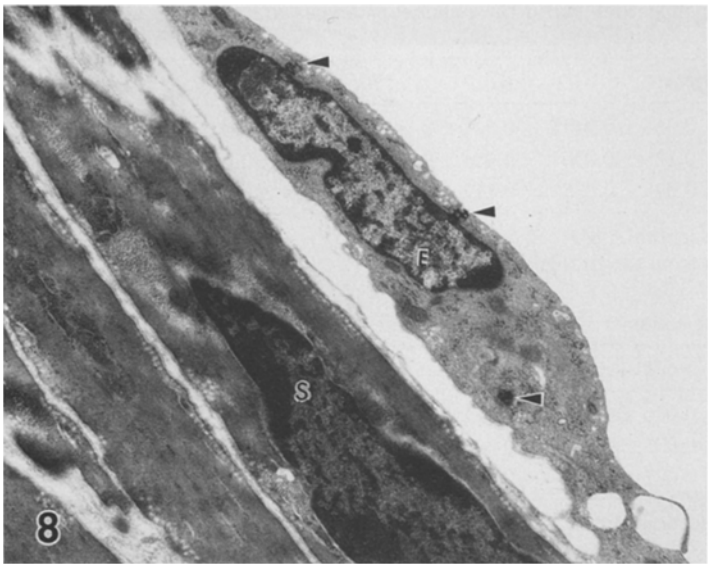


Fig. 8. Autoradiograph of pulmonary artery in bleeding to death after ^{3}H-noradrenaline administration. Silver grains *(arrows)* are observed in the pulmonary artery endothelial cell *(E)*. *S* Smooth muscle cell. × 9,800

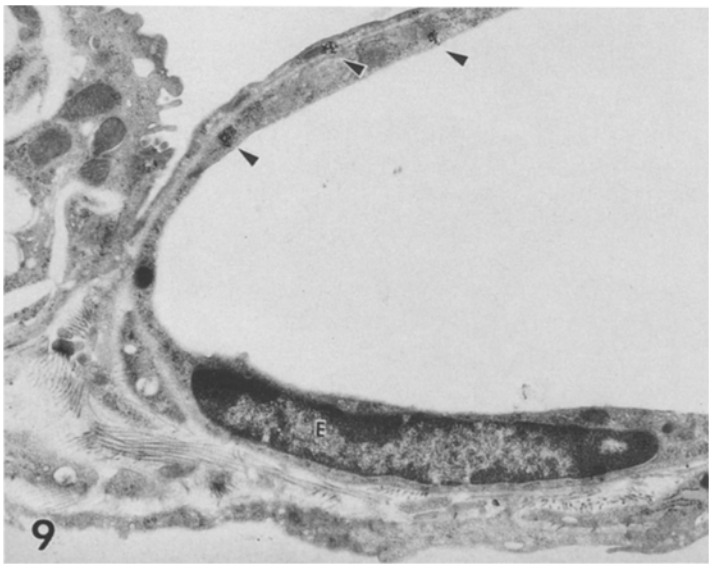


Fig. 9. Autoradiograph of pulmonary capillary in strangulation after ^{3}H-noradrenalin administration. Silver grains *(arrows)* are observed at the pulmonary capillary endothelial cell *(E)*. × 7,500

thelial cells (Figs. 7, 8, 9). In the pulmonary vein endothelial cells no silver grains were observed.

In the experimental and control groups after ^{3}H-adrenaline administration silver grain was not observed in the pulmonary blood vessel.

Discussion

Fluorescence-microscopic observations on the pulmonary blood vessels in the strangulation group and carotid-cutting group showed the specific bright green fluorescence in pulmonary artery endothelial cells. This fluorescence is suggested to represent the preserved catecholamine or serotonin of the pulmonary arteries. The author adopted the new fluorescence histochemical technique of Furness et al. (1977) which produces a water-stable fluorophore of catecholamines (especially noradrenaline). Therefore, this bright green fluorescence represents the possibility of the noradrenaline uptake by the pulmonary artery. Iwasawa et al. (1973) studied the localization of noradrenaline in the pulmonary blood vessels using fluorescence histochemical demonstration, and reported that the sites of noradrenaline uptake were the endothelium of capillaries and postcapillary venules. The author's findings are not quite in agreement with theirs, because they did not apply the new fluorescence histochemical technique of Furness et al. (1977), and so the fluorophore of catecholamines was not stable. The author applied the unlabeled PAP immunocytochemistry technique to anti-noradrenaline antibodies at the ultrastructural level, and observed the noradrenaline uptake of the pulmonary artery and capillary endothelial cells in the strangulation group and carotid-cutting group. The noradrenaline equally invaded the endothelium of the pulmonary arteries and capillaries.

The difference in the catecholamine uptake between the experimental group and the control group could not be defined from these abovementioned morphological techniques, so the author applied a radioassay technique. Animals were injected i.v. with ^{3}H-noradrenaline via the vena cava inferior, immediately after respiratory arrest. After the cardiac arrest, the pulmonary tissues were rapidly washed out with buffer to remove the extra ^{3}H-noradrenaline from the pulmonary vacular spaces. The pulmonary tissues were then fixed with the glutaraldehyde to protect the dissolving of the ^{3}H-noradrenaline uptake by the endothelial cells. The lung sections were treated by an automatic sample combustion system and counted by a liquid scintillation system. The time of administration was selected at a point of time immediately after respiratory arrest, because in the strangulation group the levels of plasma noradrenaline rapidly decreased at the intervals from the respiratory arrest to the cardiac arrest (Kita et al. 1986b). The rapid decrease of plasma noradrenaline represents the possibility of the noradrenaline uptake by the pulmonary blood vessels. In the strangulation group, significantly high levels of the pulmonary tissues' ^{3}H-noradrenaline were observed as compared with the control groups. The results of the electron-microscopic autoradiography showed the noradrenaline uptake by the endothelial cells of the pulmonary artery and capillary. Thus, in the strangulation group the volume of noradrenaline uptake by the pulmonary artery and capillary is at significantly higher levels than in the control groups.

In the strangulation group the levels of plasma adrenaline rapidly increased at the intervals from the respiratory arrest to the cardiac arrest (Kita et al. 1986b). Therefore, the author studied the possibility of adrenaline uptake by the same techniques (radioassay and autoradiography) for the noradrenaline. In the experimental and control groups after ^{3}H-adrenaline administration, the adrenaline uptake by the pulmonary blood vessels could not be observed by autoradiography. No significant differences were recognized in the volume of ^{3}H-adrenaline in the pulmonary tissues.

Buonassisi and Colburn (1980) reported that the possibility of myoendothelial junction is a pathway for transfer of molecules between endothelial cells and arterial smooth muscle cells. The author observed the many myoendothelial junctions at the site of vasoconstricted arteries, and invasive noradrenaline was clearly observed in the myoendothelial junction. Thus, the invasive noradrenaline of the endothelial cells may be passed on to the underlying smooth muscle cells. In the case of the blood vessels with a thin muscular coat or without muscular coat, there are many discussions about the possibility of the endothelial contractility (Hammersen 1980). In asphyxia, pulmonary capillaries and arterioles contract and take up noradrenaline. From this it appears that the invasive noradrenaline participates in the endothelial contractility.

The mechanism of the pulmonary vasoconstriction in strangulation is due to the fact that the plasma noradrenaline, increased by asphyxia, invades the endothelial cells of pulmonary arteries and capillaries and causes the vasoconstriction.

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References

Al-Ubaidi F, Bakhle YS (1980) Metabolism of vasoactive hormones in human isolated lung. Clin Sci 58:45–51

Boileau JC, Campeau L, Biron P (1971) Comparative pulmonary fate of intravenous epinephrine. Rev Can Biol 30:281–286

Boileau JC, Crexells C, Biron P (1972) Free pulmonary passage of dopamine. Rev Can Biol 31:69–72

Buonassisi V, Colburn P (1980) Hormone and surface receptors in vascular endothelium. Adv Microcirc 9:76–94

Corrodi H, Hillarp NA, Jonsson G (1964) Fluorescence methods for the histochemical demonstration of monoamines. 3. Sodium borohydride reduction of the fluorescent compounds as a specificity test. J Histochem Cytochem 12:582–586

Falck B, Hillarp NA, Thieme G, Torp A (1962) Fluorescence of catecholamines and related compounds condensed with formaldehyde. J Histochem Cytochem 10:348–354

Furness JB, Costa M, Wilson AJ (1977) Water-stable fluorophores, produced by reaction with aldehyde solutions, for the histochemical localization of catechol- and indolethylamines. Histochemistry 52:159–170

Gillis CN, Cronau LH, Mandel S, Hammond GL (1979) Indicator dilution measurement of 5-hydroxytryptamine clearance by human lung. J Appl Physiol 46:1178–1183

Ginn R, Vane JR (1968) Disappearance of catecholamines from the circulation. Nature 219: 740–742

Hammersen F (1980) Endothelial contractility – Does it exist? Adv Microcirc 9:95–134

Hughes J, Gillis CN (1968) Uptake of ^{3}H-norepinephrine by perfused rat lung. Fed Proc: 467
Hughes J, Gillis CN, Bloom FE (1969) The uptake and disposition of dl-norepinephrine in perfused rat lung. J Pharmacol Exp Ther 169:237–248
Iwasawa Y, Gillis CN, Aghajanian G (1973) Hypothermic inhibition of 5-Hydroxytryptamine and norepinephrine uptake by lung: Cellular location of amines after uptake. J Pharmacol Exp Ther 186:498–507
Joftes DL, Warren S (1955) Simplified liquid emulsion radioautography. J Biol Photogr Assoc 23:145–150
Kita T, Shigezane J, Furuya Y (1986a) Mechanism of the pulmonary congestion in ligature strangulation (1). Jpn J Legal Med 40:129–131
Kita T, Shigezane J, Furuya Y (1986b) Mechanism of the pulmonary congestion in ligature strangulation (2). Jpn J Legal Med 40:282–286
Kjellström T, Ahlman H, Dahlström A, Hansson GK, Risberg B (1984) The uptake of 5-hydroxytryptamine in endothelial cells cultured from the pulmonary artery in rats. Acta Physiol Scand 120:243–250
Onteniente B, Geffard M, Calas A (1984) Ultrastructural immunocytochemical study of the dopaminergic innervation of the rat lateral septum with anti-dopamine antibodies. Neuroscience 13:385–393
Prokop O, Göhler W (1976) Forensische Medizin. 3. Aufl. Fischer, Stuttgart New York
Strum JM, Junod AF (1972) Radioautographic demonstration of 5-hydroxytryptamine ^{3}H uptake by pulmonary endothelial cells. J Cell Biol 54:456–467

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