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Effects of vasodilators on fibrin-induced pulmonary edema, so-called neurogenic pulmonary edema, in the rat

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Abstract: The present study was undertaken to evaluate the effects of vasodilators on the development of neurogenic pulmonary edema. Pulmonary edema was induced by injecting fibrinogen and thrombin into the cisterna magna of vagotomized rats (fibrin-induced pulmonary edema). Before the intrathecal injections, rats were pretreated with intravenous injection of one of the following vasodilators: phentolamine, isoproterenol, nifedipine, diltiazem, isosorbide dinitrate, or substance P. Each vasodilator reduced the incidence of fibrin-induced pulmonary edema and lung-water ratio dose-dependently except nifedipine and diltiazem. There was a uniform relationship between the lung-water ratio and the prefibrin mean arterial pressure obtained under administration of different doses of each drug. A similar relationship was obtained even if the drug used was different. Treatment with nifedipine or diltiazem, however, diminished the blood pressure but provided less protection against the development of pulmonary edema. The blood volume in edema-positive lungs was minimally different from that in edema-negative lungs. These results suggest that the neurogenic pulmonary edema may be effectively prevented by most vasodilators except Ca^{++}-blockers.

Key words: Neurogenic pulmonary edema, Vasodilators, Fibrin, Lung-water ratio, Lungs, Ca^{++}-blockers

Introduction

Neurogenic pulmonary edema sometimes occurs in central nervous diseases such as cervical vertebral injuries and cerebral hemorrhage. This condition is characterized by enhanced sympathetic nerve activity [1], and therefore its etiology has been attributed to an increased lung capillary pressure which is evoked by a blood volume shift from the systemic to the pulmonary circulation as well as by pulmonary venoconstriction [2,3]. The increased capillary pressure may enhance the transvascular filtration and may possibly cause focal endothelial damage [4,5].

Slotman et al. [6], Demling et al. [7], and Foulke et al. [8] reported that vasodilators are effective in preventing experimentally induced permeability pulmonary edema with intravenous infusion of oleic acid and endotoxin. These investigators suggested that the vasodilators caused the vasodilatation of the systemic vasculature as well as the pulmonary vascular beds, diminishing the change in pulmonary capillary pressure, and decreasing the transvascular filtration rate. For the neurogenic pulmonary edema, however, no systematic study has been performed. As a model of neurogenic pulmonary edema, we used fibrin-induced pulmonary edema caused by injecting fibrinogen and thrombin into the cisterna magna of the rat [9–12].

Materials and methods

The study was performed in accordance with the guidelines for animal experimentation of Nagoya University.

A model of neurogenic pulmonary edema

Fifty-nine rats of either sex, weighing 190–350 g, were anesthetized with pentobarbital sodium (35 mg/kg i.p.). Midcervical tracheotomy was performed and an endotracheal tube was inserted. The right femoral artery was cannulated for measurements of systemic arterial pressure and heart rate, and the femoral vein for venous injection of drugs, respectively. Bilateral midcervical vagal nerves were severed before injection of fibrinogen and thrombin. After the surgical procedure, the rat was fixed on a stereotaxic apparatus.

Fibrinogen (Midori Juji, Osaka, Japan) and thrombin (Mochida Pharmaceutical, Tokyo, Japan) were dis-

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solved in saline at concentrations of 100 mg/ml and 200 U/ml, respectively. These solutions, 0.05 ml each, were injected successively into the cisterna magna of individual rats. When froth appeared in the tracheal tube, the experiment was stopped and the animal was quickly killed to excise the lungs; otherwise, the experiment was terminated after full term of 10 min. Wet lung weight was measured. Then lungs were dried at 70°C for 2 days to obtain the dry lung weight. The following two criteria were used to determine whether pulmonary edema occurred: a gross finding of edema froth in the tracheal tube or bronchi without compression, and a measurement of lung-water ratio [(wet lung weight—dry lung weight)/dry lung weight] larger than 4.6 [11]. No discrepancy was noted between two judgments performed independently on the basis of the two criteria.

Blood volume in the lungs was estimated in eight edema-positive lungs of control rats (saline-pretreated and fibrin-injected rats) as well as in five normal lungs according to Holcroft and Trunkey [13]. Briefly, after opening the chest, lung lobes were clamped at both hili and excised, and blood was collected from the right atrium. Lung lobes were homogenized after addition of an equal weight of water. The blood, homogenate, and supernatant were weighed and dried at 70°C for 2 days. Water content in blood, homogenate and supernatant were calculated as a ratio of [(wet weight—dry weight)/dry weight]. Hemoglobin concentrations in blood and supernatant were measured with a Hemokit (Nihon Shoji, Osaka, Japan) to estimate the weights of blood and total water in the lungs.

Drugs used

Rats were pretreated intravenously with saline or agent, 5 min before the fibrin treatment. The dose of each drug solution was chosen so that the change in pressure measured just before the fibrin treatment was greater than 10 mmHg. Drugs studied were phentolamine (Regitine, Ciba-Geigy, Hyogo, Japan), isoproterenol bitartrate (Sigma Chemical, St. Louis, Mo.), nifedipine (Adalat, Takeda Pharmaceutical, Osaka, Japan), isosorbide dinitrate (Nitrol, Eisai, Tokyo, Japan), diltiazem (Herbesser, Tanabe, Osaka, Japan), and substance P (Peptide Institute, Osaka, Japan). Nifedipine was dissolved with ethanol at a concentration of 1 mg/ml and was diluted with saline before use.

Statistical analyses

We performed a one-way analysis of variance (ANOVA) or paired *t*-test. Statistical significance of the difference between two means in the analysis of variance was examined by Scheffe's method for multiple comparisons [14]. For nominal parameters such as incidence of pulmonary edema, a median test was performed. The level of significance was taken as 0.05.

Table 1. Lung water content

	n	Blood weight (g)	Wet lung weight (g)	Dry lung weight (g)
PE(−)	(5)	0.30 ± 0.04	1.63 ± 0.12	0.35 ± 0.05
PE(+)	(8)	0.38 ± 0.04	2.73 ± 0.28**	0.42 ± 0.05

PE(−) and PE(+) indicate the control lungs without any treatment and edema-positive lungs obtained with intrathecal injections of fibrinogen and thrombin.
** $P < 0.01$ compared with PE(−) group.

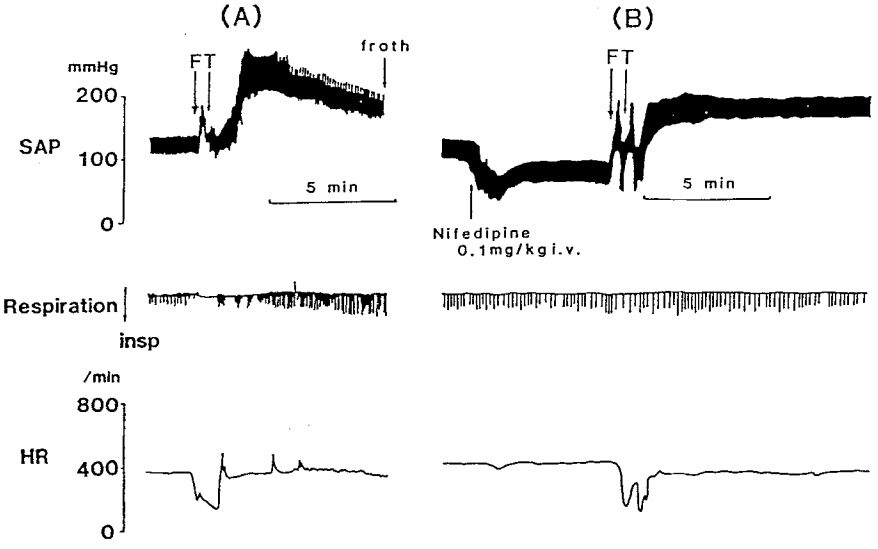

Fig. 1a,b. Two typical recordings of systemic arterial pressure (*SAP*), respiratory movement and heart rate (*HR*) in a saline-treated rat (**A**) and nifedipine-treated rat (**B**). *Thick* and *thin arrows* indicate the intrathecal injections of fibrinogen (*F*) and thrombin (*T*), respectively. In the recordings of respiration, downward movement indicates inspiration

Results

Fibrin-induced pulmonary edema

Intracisternal injection of fibrinogen caused a first transient rise of systemic arterial pressure, and subsequent injection of thrombin caused a sustained greater increase (Fig. 1). The respiratory movement was irregular, intermingled with apnea or Cheyne-Stokes rhythm. In all saline-treated rats, pink-colored edema froth appeared in the tracheal tube within 6 min after the administration of fibrin. The wet lung weights obtained were significantly higher than those in the fibrin-untreated rats ($P < 0.01$; ANOVA), whereas the dry lung weight was not different (Table 1). The blood volume in the lungs was also increased by fibrin treatment, but the difference between the blood weights obtained in edema-positive and edema-negative lungs was very small (0.08 g), as shown in Table 1.

Effects of vasodilators on fibrin-induced pulmonary edema

As shown in Table 2, the incidence of pulmonary edema was significantly ($P < 0.05$; ANOVA) reduced by each vasodilator except diltiazem. The effective dose of each vasodilator used was as follows: 1 mg/kg of phentolamine, 0.01 mg/kg of isoproterenol, 0.1 mg/kg of nifedipine, 0.3–1 mg/kg of isosorbide dinitrate, and 0.01 and 0.1 mg/kg of substance P. Dose-dependent inhibitory effects were observed with phentolamine and substance P. The lung-water ratio was reduced by most vasodilators ($P < 0.05$, ANOVA), except nifedipine and diltiazem. The treatment with diltiazem in particular did not reduce the incidence of pulmonary edema even at a dose of 10 mg/kg which was sufficient to decrease the systemic arterial pressure by more than 50 mmHg.

Relationship between lung-water ratio and systemic arterial pressure

Each vasodilator lowered the systemic arterial pressure (prefibrin treatment) significantly, as shown in Table 3 ($P < 0.05$; ANOVA), and the systemic arterial pressure was increased by intracisternal injections of fibrinogen and thrombin. Figure 2 shows the relationship between the prefibrin systemic arterial pressure and lung-water ratio. With all agents, as the prefibrin arterial pressure was decreased, the lung-water ratio was also decreased. There was a uniform S-shaped relationship for most vasodilators except for the Ca^{++}-blockers, nifedipine and diltiazem. The relationship obtained for Ca^{++}-blockers was obviously shifted leftward to that obtained for the other agents. There was no marked relationship between the post-fibrin peak systemic arterial pressure and lung-water ratio (data not shown).

Discussion

The results obtained in the present study showed that the vasodilators prevented the neurogenic pulmonary edema at the doses which reduced the systemic arterial pressure by more than 10 mmHg. Although the vasodilators studied had a different mechanism of action in relaxing the vascular smooth muscle, there was a clear correlation between the lung-water ratio and the prefibrin systemic blood pressure. If is possible that the preventive action of vasodilators on neurogenic pulmonary edema is associated with their vasodilating action.

Table 2. Incidence of pulmonary edema and lung-water ratio

Agents (mg/kg)	n	Incidence (%)	Wet lung weight (g)	Lung-water ratio (g)
Saline	8	100	2.73 ± 0.28	6.40 ± 0.27
Phentolamine				
(0.05)	6	60*	2.23 ± 0.66	5.23 ± 0.79
(1.0)	5	0***	1.56 ± 0.01	3.94 ± 0.23**
Nifedipine				
(0.1)	7	57*	2.46 ± 0.40	5.47 ± 0.66
Diltiazem				
(1–10)	5	100	2.14 ± 0.57	5.35 ± 1.28
Isoproterenol				
(0.01)	5	40*	2.15 ± 0.56	4.43 ± 0.60*
Isosorbide dinitrate				
(0.3–1)	9	67**	2.61 ± 0.36	4.92 ± 0.31*
Substance P				
(0.01)	5	20**	1.55 ± 0.34	4.08 ± 0.65*
(0.1)	5	0***	1.48 ± 0.14	3.60 ± 0.12**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ *vs* saline.

Table 3. Changes in systemic arterial pressure (SAP)

Agents (mg/kg)	Change in SAP caused by agent (mmHg)	Change in SAP caused by fibrin (mmHg)
Saline	–	74.8 ± 4.2
Phentolamine		
(0.05)	−12.6 ± 3.7*	67.8 ± 8.5
(1.0)	−31.8 ± 6.9**	86.8 ± 9.7
Nifedipine		
(0.1)	−35.7 ± 3.6***	81.3 ± 4.8
Diltiazem		
(1–10)	−27.2 ± 4.6**	80.8 ± 4.8
Isoproterenol		
(0.01)	−16.8 ± 5.0*	86.2 ± 6.4
Isosorbide dinitrate		
(0.3–1)	−12.8 ± 3.7**	68.0 ± 7.2
Substance P		
(0.01)	−21.0 ± 3.1**	73.2 ± 9.4
(0.1)	−19.0 ± 3.4**	79.0 ± 6.6

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

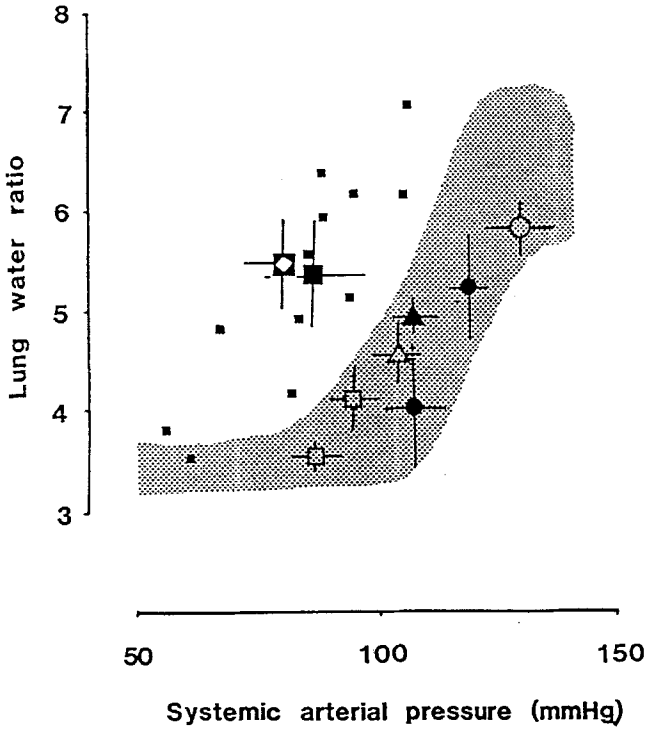

Fig. 2. Relationships between lung-water ratio and systemic arterial pressure obtained before the intrathecal injection of fibrinogen and thrombin. *Shaded area* indicates the area where all relations were included except those of nifedipine and diltiazem. Each *small square* indicates the relation obtained under the treatment of nifedipine and diltiazem. *Open circle*, saline; *closed circle*, phentolamine; *open triangle*, isoproterenol; *closed triangle*, isosorbide dinitrate; *open square*, substance P; *closed square*, diltiazem and nifedipine

Sarnoff and Sarnoff [10] ascribed the development of neurogenic pulmonary edema, such as fibrin-induced pulmonary edema, to a shift of blood volume from the systemic to the pulmonary circulation, suggesting an increase in pulmonary capillary pressure which might enhance the transvascular filtration rate of plasma. Therefore, the mechanism by which vasodilators prevent neurogenic edema may be related to a decrease in the blood volume shift from the systemic circulation to the pulmonary by dilating the systemic vascular beds. However, the calculated blood content in lungs was not significantly altered by the occurrence of neurogenic pulmonary edema, suggesting that the blood volume shift from the systemic circulation was not a major factor in the development of neurogenic pulmonary edema.

Vasodilators have been proven to be effective in preventing permeability pulmonary edema induced by endotoxin or oleic acid [6–8]. In awake animals, however, Bishop et al. [15] demonstrated that the vasodilation induced by minoxidil increased blood flow to the injured area via a reflex-increase in cardiac output but did not resolve the injury. Such a reflex-increase in cardiac output could theoretically increase the pulmonary microvascular pressure, accelerating the formation of edema. However, this is not the case in neurogenic pulmonary edema; because the sympathetic nerve activity has already been enhanced [1], the sympathetic nerve activity is minimally altered on the basis of enhanced status.

The results of the present study showing that the relationship between the lung-water ratio and systemic arterial pressure was similar among the different vasodilators suggested that the sites of action of these vasodilators were also the same. Because of the difficulty of assessing the pulmonary hemodynamics in the rat, we could not ascertain the site of action of vasodilators in preventing neurogenic pulmonary edema. Some investigators [6–8] who studied the preventive action of vasodilators on permeability pulmonary edema in the dog ascribed the preventive effects to the dilatation of pulmonary veins, which could reduce the intracapillary pressure.

Failure of Ca^{++}-blockers to prevent the neurogenic pulmonary edema to the same extent as the other vasodilators might provide a clue as to the common site of action. Alternatively, the site may be characterized by a reaction which is resistant to Ca^{++}-blockers. The Ca^{++}-influx may not be an important pathway for the development of fibrin-induced pulmonary edema. Most investigators suggested that the vascular permeability may be elevated by the contraction of vascular endothelial cells. Johnson et al. [16] demonstrated that several protein kinase C inhibitors prevented the enhancement of lung vascular permeability caused by neutrophils activated with dioctanoylglycerol, a second messenger of protein kinase C activation, in isolated perfused guinea pig lungs. In conjunction with phosphatidylserine and intracellular Ca^{++}, diacylglycerols activate the protein kinase C. Therefore, the roles of second messengers including Ca^{++} as well as cyclic nucleotides, diacylglycerol and inositol triphosphate, must be investigated further.

It is still unknown whether the pulmonary endothelial cells are more sensitive to extracellular Ca^{++} concentration compared to other vascular endothelial cells. Another possible site may be the pulmonary artery and/or vein, which, in contrast with systemic vascular smooth muscles, may be resistant to Ca^{++}-blockers. An example of such a difference between the systemic and pulmonary vasculature is shown in our laboratory [17]: alpha-human natriuretic polypeptide caused a relaxation of pulmonary artery and vein in the dog, and this effect was partly dependent on the extracellular Ca^{++} concentration, whereas the systemic vascular strips were not relaxed in response to alpha-human natriuretic polypeptide. At the same time, we reported that canine

pulmonary artery and vein were resistant to Ca^{++}-blockers, whereas the systemic vascular strips were relaxed.

In summary, vasodilators appear to be effective in preventing the neurogenic pulmonary edema, possibly via their action at a common site characterized by its resistance to Ca^{++}-blockers.

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