S1-Receptor Participation in Serotonin-Induced Pulmonary Edema in the Dog†

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Abstract—A canine lung-perfusion preparation was used to evaluate the role of serotonin receptor subtype in the development of serotonin-induced pulmonary edema. Ketanserin, an S2-receptor antagonist, blocked an increase in pulmonary arterial pressure caused by serotonin, but not the development of pulmonary edema. Methysergide, an S1- and S2-receptor antagonist, prevented the increase in pulmonary arterial pressure and edema formation caused by serotonin. These results suggest that the S1-receptor may participate in the development of pulmonary edema.

Histamine and serotonin are vasoconstrictors in the pulmonary circulation and possibly may alter fluid equilibrium across the microvascular membranes via a change in capillary pressure. Histamine may increase the pulmonary capillary permeability (1), but recent studies failed to demonstrate such a phenomenon (2). Vasoconstrictor effects of histamine are generally confined to the postcapillary side, but those of serotonin confined to the precapillary side (3). Because of such a difference in the vasoconstrictor effects between histamine and serotonin, there is a controversy with regard to how histamine and serotonin may affect the plasma filtration across the pulmonary vasculature.

The present study was undertaken to evaluate the effects of serotonin on the development of pulmonary edema, with respect to the so-called S-receptor subtypes, using a lung-perfusion preparation.

Twenty-five mongrel dogs, weighing 8–12 kg, were used. They were anesthetized with intravenous injections of 400 mg/kg of urethane and 40 mg/kg of alpha-chloralose. For priming of the extracorporeal circuit, larger dogs were exsanguinated. Collected blood was treated with 5000 units of heparin.

After intubation, lungs were artificially ventilated with room air at a respiratory rate of 16 times/min and a tidal volume of 20 ml/kg. End-expiratory pressure was maintained at 2 cmH2O. After opening the chest, all of the cardiopulmonary nerves were severed to avoid a spontaneous occurrence of pulmonary edema (4). Both the right and left atria and pulmonary arterial trunk were cannulated. The lung-perfusion preparation was made after steady perfusion status was obtained in a right-heart-bypass preparation. Blood was pumped from a reservoir to the pulmonary artery. The temperature of blood was kept at 38°C in the venous reservoir. The height of the outlet for venous return from the left atrium was adjusted, above the blood level in the reservoir, to keep the left atrial pressure constant at any desired level. Perfusion flow was maintained constant at 60 ml/min/kg throughout the experiment with the aid of a roller pump (MERA, MPS-15R). Pulmonary arterial and left atrial pressures were measured by pressure transducers (Nihon Kohden, LPU-0.1). A change in reservoir blood volume was also measured as a change in hydrostatic pressure in a cylindrical blood reservoir by another pressure transducer.

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Twenty-five dogs were divided into three groups, 5 in the control, 5 for the treatment with histamine (Histamine hydrochloride, Sigma) and 15 with serotonin (5-Hydroxytryptamine creatinine sulfate, Sigma). In serotonin-infused dogs, 5 dogs were pretreated with 10 mg of ketanserin (Sandoz) and another 5 dogs treated with 30 mg of methysergide (Sandoz), followed by the serotonin infusion 10 min later. Histamine or serotonin was infused into a tube between the blood reservoir and pulmonary arterial cannula (100 μg/min). Ketanserin and methysergide were injected into the blood reservoir and mixed with blood in the reservoir (approx. 500 ml). Left atrial pressure was usually maintained at 5 cmH₂O. Before and during the infusion, left atrial pressure was changed upwards and downwards between 5 and 15 cmH₂O, 5 cmH₂O each step. And 5 min were allowed to elapse between each step. In each preparation, experiments were completed in 90 min.

At the end of the experiment, extravascular lung water and dry weights (EVLW and EVDW) were estimated, following the methods of Holkroft and Trunkey (5). Briefly, wet and dry weights of blood, homogenate of lungs and its supernatant were measured before and after drying at 70°C for two days, respectively. Hemoglobin concentrations in the blood and supernatant were measured with a kit (Nippon Shoji, Hemo Kit-S). Then, the weights of the blood in the lungs, EVLW and EVDW were calculated, assuming that hemoglobin in red cells remained in the intravascular space.

Data were expressed as the mean and S.E. The significance of the difference between two means was determined by Student’s t-test. The level of significance was taken as 0.05.

Elevating the left atrial pressure caused a stepwise increase in the rate of reservoir blood loss, pulmonary arterial pressure and peak airway pressure (Fig. 1). Serotonin enhanced the rate of reservoir blood loss and elevated the pulmonary arterial and peak

![Graph](image-url)

**Fig. 1.** Effects of changing the left atrial pressure (LAP) on rate of reservoir blood loss, pulmonary arterial pressure and peak airway pressure. Open circle, before the infusion of serotonin and the administrations of ketanserin or methysergide (n=5); open triangle, during the infusion of serotonin at 100 μg/min into a tube between the blood reservoir and pulmonary arterial cannula (n=5); closed circle, during the infusion of serotonin under pretreatment with 10 mg of ketanserin (n=5); closed triangle, during the infusion of serotonin under pretreatment with 30 mg of methysergide (n=5). *, ** and ***: P<0.05, P<0.01 and P<0.001, respectively, vs. control; † and ††: P<0.05 and P<0.01, respectively, vs. ketanserin.
airway pressures (P<0.01). Histamine also significantly elevated the rate of reservoir blood loss (P<0.05) and the peak airway pressure (P<0.01) up to 0.209±0.036 ml/min·kg⁻¹ and 15.1±1.2 cmH₂O (n=5), respectively, at 5 cmH₂O of left atrial pressure. Pulmonary arterial pressure was elevated to a much smaller extent (22.4±1.6 cmH₂O, n=5).

Both ketanserin and methysergide affected neither the rate of reservoir blood loss, pulmonary arterial pressure nor peak airway pressure in all 5 dogs in each group. Ketanserin completely blocked the increase in pulmonary arterial pressure caused by serotonin, and it slightly diminished the increase in the rate of reservoir blood loss and peak airway pressure. Nevertheless, values of the latter two parameters were significantly larger than those in the control, except at 5 cmH₂O of left atrial pressure (P<0.05). On the other hand, methysergide completely blocked all of the increases in the three parameters caused by serotonin.

Ratios of EVLW to EVDW (EVLW/EVDW) obtained in the five groups are shown in Fig 2. An increase in the ratio was caused by serotonin. It was not blocked by ketanserin, but by methysergide. The weights of blood in the lungs were not different among the three groups treated with serotonin: 15.5±4.3, 13.0±2.2 and 11.9±1.7 g/kg B.W. in the serotonin, ketanserin and methysergide groups, respectively.

Ketanserin, an α₁-adrenoceptor antagonist (6), is known also as a pure S₂-receptor antagonist (7), whereas methysergide blocks both S₁- and S₂-receptors at high doses (8). Both ketanserin and methysergide blocked the increase in pulmonary arterial pressure, suggesting participation of S₂-receptor in the vasoconstriction. A difference in EVLW/EVDW between the ketanserin- and methysergide-pretreated dogs seemed to correspond to the difference in receptors which were occupied by these antagonists. It is likely that the S₁-receptor mediates the serotonin-induced pulmonary edema, because the EVLW/EVDW values obtained under the pretreatment with ketanserin were still larger than those with methysergide or in the controls.

De Clerck et al. (9) showed that ketanserin inhibited a change in vascular permeability induced by serotonin in rat skin, suggesting that vascular permeability was elevated via the S₂-receptor. However, Rippe et al. (3) showed that vascular permeability in canine lungs was not changed by serotonin infused at an infusion rate of 20–60 μg/min. Even if the vascular permeability in lungs was elevated via either S₁- or S₂-receptor, the simultaneous activations of both receptors were thought to block such an elevation. Actually, there is a possibility that the S₁-receptor may mediate the elevation of vascular permeability in canine lungs, since in the ketanserin-pretreated group, pulmonary edema occurred without the increase in pulmonary arterial pressure. The contribution of each serotonin-receptor subtype to the vascular permeability in lungs is still to be investigated.

It is still difficult to confine the site of action of serotonin in the development of pulmonary edema. A part of the reservoir blood loss may reflect blood loss via the broncho-pulmonary anastomoses through the systemic circula-
tion (10). Besides the pulmonary vascular wall, the bronchial vascular beds are other possible sites for the plasma filtration, as shown by Pietra et al. (11). Possibly, the blood flow distribution in the lungs may have been altered by serotonin, since the rate of reservoir blood loss was more increased in the serotonin group than in the ketanserin-pretreated group, in spite of the similar values in EVLW/EVDW. Blood flow from the pulmonary circulation to the bronchial circulation may be increased by the elevation of pulmonary capillary pressure induced by the pulmonary vasoconstriction as has been indicated with histamine (3).

A possible participation of $S_1$-receptors, in the increase in peak airway pressure has been suggested as well as that of $S_2$-receptors, in the present study: i.e., the increase in peak airway pressure was blocked partly by ketanserin and the remaining part, by methysergide. Bronchoconstriction caused by serotonin was reported to be mediated by $S_2$-receptor (5, 12). How the $S_1$-receptor mediates the increase in peak airway pressure is still unknown.

References
1 Brigham, K.L. and Owen, P.J.: Increased sheep lung vascular permeability caused by histamine. Circ. Res. 37, 647–657 (1975)
2 Drake, R.E. and Gabel, J.C.: Effect of histamine and alloxan on canine pulmonary vascular permeability. Am. J. Physiol. 239, H96–H100 (1980)
3 Rippe, B., Allison, R.C., Parker, J.C. and Taylor, A.E.: Effects of histamine, serotonin, and nor-epinephrine on circulation of dog lungs. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 57, 223–232 (1984)
4 Kainuma, M., Ichimura, I., Shigei, T. and Ishikawa, N.: Coronary flow in heart-lung perfusion affected by cardiopulmonary nerves and $\text{PaCO}_2$. Am. J. Physiol. 249, H995–H1000 (1985)
5 Holcroft, J.W. and Trunkey, D.D.: Extravascular lung water following hemorrhagic shock in the baboon. Am. J. Surg. 180, 408–417 (1974)
6 McCall, R.B. and Schuette, M.R.: Evidence for an alpha-1 receptor-mediated central sympatheinhibitory action of ketanserin. J. Pharmacol. Exp. Ther. 228, 704–710 (1984)
7 Van Neuten, J.M., Janssen, P.A. and Vanhoutte, P.M.: Pharmacological properties of serotonergic responses in vascular, bronchial and gastrointestinal smooth muscle. In Vascular Neuro-effector Mechanisms, 4th International Symposium, Edited by Bevan, J.A., Fujiwara, M., Maxwell, R.A., Mohri, K., Shibata, S. and Toda, N., Raven Press, New York (1983)
8 Leysen, J.E., Awouters, F., Kennis, L., Laduron, P.M., Vanderbeek, J. and Janssen, P.A.: Receptor binding profile of R41468, a novel antagonist at 5-HT$_2$ receptors. Life Sci. 28, 1015–1022 (1981)
9 De Clerck, F., Van Gorp, L., Beetens, J. and Reneman, R.S.: Platelet-mediated vascular permeability in the rat: A predominant role for 5-hydroxytryptamine. Thromb. Res. 38, 321–339 (1985)
10 Nakamura, J., Zhang, S. and Ishikawa, N.: Role of pulmonary innervation in canine in-situ lung perfusion preparation—A new model of neurogenic pulmonary edema. Clin. Exp. Pharmacol. Physiol. 14, 23–30 (1987)
11 Pietra, G.G., Szidon, J.P., Leventhal, M.M. and Fishman, A.P.: Histamine and interstitial pulmonary edema in the dog. Circ. Res. 29, 323–337 (1971)
12 Saxena, P.R. and Lawang, A.: A comparison of cardiovascular and smooth muscle of 5-hydroxytryptamine and 5-carboxamidotryptamine, a selective agonist of 5-HT$_1$ receptors. Arch. Int. Pharmacodyn. Ther. 277, 235–252 (1985)