hematocrit increased with age in the cyanotic group ($P < 0.01$) but not in the control group. The RV/LV ratio remained unchanged with age. The RV and LV wall thicknesses, respectively, increased with age in both the cyanotic and control groups (each $P < 0.05$). The heart rates remained unchanged with age in both groups. A significant degree of acidosis was found in the 4-week-old but not in the 8-week-old cyanotic rats.

Age-related changes in flow heterogeneity in the cyanotic and control groups are shown in Table 3. No significant age-dependent trend was found, although the CV tended to increase in LV myocardium in the cyanotic group ($P = 0.2$). In addition, no difference was observed in the CV of either the RV or LV myocardium between the 4- and 8-week-old control rats.

### Effect of reoxygenation

The reoxygenation group (normally ventilated cyanotic rats) showed arterial $PaO_2$ and $SaO_2$ values that were similar to those observed in the control group (Table 2). Blood pressure tended to decrease after induction of normoxic ventilation (103 ± 18 vs. 88 ± 22 mmHg, $P = 0.14$); and the heart rate was not changed by the ventilatory setting (353 ± 39 vs. 360 ± 28 beats/min, $P = 0.68$).

When the cyanotic myocardium was reoxygenated, the CV became markedly smaller than in that in either ventricle in the cyanotic and control groups at 8 weeks of age (Table 3), although the difference was not statistically significant at 4 weeks of age. The CV of the 8-week LV in the reoxygenation group tended to be lower than that in the control group.

### Left versus right ventricles

No difference was found in CV between the LV and RV myocardium although there was a trend toward a higher CV in RV myocardium than in LV myocardium in the 4-week-old reoxygenated and 8-week-old control groups (Table 3).

## Discussion

When the heart is exposed to acute hypoxia, regional myocardial flow may become less heterogeneous as a response to an altered $O_2$ demand/supply balance,[5] thus leading to the hypothesis that local vascular control functions to improve the efficiency of regional $O_2$ delivery under hypoxia as a self-protective mechanism. The question raised in this study is whether the neonatal/infantile myocardium that is exposed to chronic hypoxia shows a similar level of flow heterogeneity observed under acute hypoxia and how the flow heterogeneity changes when exposed to a normoxic environment. The interest stems from the facts that patients with many types of congenital heart disease have cyanosis from birth because of pulmonary stenosis/atresia or an intracardiac mixing and that once intracardiac repair is performed the myocardium is suddenly exposed to a normoxic condition. This is the first study to investigate regional myocardial flow heterogeneity in a neonatal animal model mimicking the clinical sequence of cyanotic congenital heart diseases.

The new findings are that (1) myocardial blood flow heterogeneity in 4-week hypoxic hearts did not differ from that observed in age-matched control hearts in either the LV or the RV despite having significant somatic growth retardation and homeostatic changes (i.e., high hemoglobin, metabolic acidosis, RV hypertrophy). (2) Eight weeks of chronic hypoxia slightly increased the heterogeneity of regional myocardial flows in both the LV and RV. This tendency was noticeable in LV myocardium although no age-related changes were observed in myocardial blood flow heterogeneity. (3) Reoxygenation resulted in decreased flow heterogeneity in both the LV and RV in the 8-week hypoxic hearts.

### Effect of chronic hypoxia on myocardial blood flow heterogeneity

The speculation was that myocardial blood flow could be less heterogeneous under chronic hypoxia than under normoxia. This was based on the fact that acute hypoxia leads to a decrease in regional myocardial flow heterogeneity. However, regional myocardial flow in chronically hypoxic myocardium was either similar to or highly heterogeneous in comparison to that of control normoxic myocardium. Even a slight increase in flow heterogeneity was observed in the 8-week cyanotic rats.

We speculated that this discrepancy between the present chronic and acute hypoxic models would result partly from their differences of vascular regulatory capacity. In the present study, we used the immature young rat raised in a hypoxic chamber from birth, whereas Matsumoto et al. used the mature adult rabbit raised normally.[5] In the heart under acute hypoxia, vascular regulation plays a contributing role in reducing flow heterogeneity probably through local coordination of vascular tone.[5] Such locally coordinated vasoregulation might not function under chronic exposure to hypoxia. Furthermore, coronary vascular anatomy and basal oxidative metabolism under chronic hypoxia, which differs from those in the normal heart,[12,13] may influence flow heterogeneity. That is, it is possible that these determining factors of flow heterogeneity were

modified under chronic exposure to hypoxia and resulted in similar or higher flow heterogeneity compared with that in the normoxic heart. Further studies are necessary for a better understanding of the influences of chronic hypoxia on these determinant factors of myocardial flow heterogeneity.

Erythrocytosis accompanying chronic hypoxia may also have an increasing effect on flow heterogeneity. Increased blood viscosity and hematocrit associated with erythrocytosis increase red blood cell (RBC) aggregation and the frequency of small vessel occlusion.[14–16] RBC aggregation occurs with higher frequency in low-flow regions and increases the frequency of the momentary blocking of microvessels preferably in low-flow regions,[17,18] thereby increasing flow heterogeneity.

### Effect of reoxygenation on myocardial blood flow heterogeneity

The vasoresponse to reoxygenation is responsible for the decreased myocardial flow heterogeneity. Daniell and Bagwell showed that a sudden increase in $FiO_2$ (from 0.25 to 1.0) resulted in coronary flow decrease with decreased isometric systolic tension in open-chest dogs,[19] implying that the $FiO_2$ increase induced coronary vasoconstriction. The slight drop in blood pressure observed in this study when the ventilator setting changed indicates that reoxygenation may induce coronary constriction even in the heart exposed to chronic hypoxia.

The effect of reoxygenation or coronary constriction on flow heterogeneity, however, would be dependent on the degree of coronary tone under hypoxia. In the heart exposed to acute hypoxia, flow heterogeneity decreased, presumably owing to local vasoregulation.[5] However, regional myocardial flow shows a higher level of heterogeneity under more severe $O_2$ deprivation (anoxia) than under normoxia.[4,7] That is, the response of myocardial flow heterogeneity to acute hypoxia is biphasic according to the degree of $O_2$ supply deficiency. During the phase of reduced flow heterogeneity under acute hypoxia, the vascular tone is locally coordinated.[5] On the other hand, during the phase of increased flow heterogeneity, coronary vessels may be close to the maximum dilated condition because the flow heterogeneity under anoxia is similar to that in the heart at the adenosine-induced maximum vasodilatory state. Considering that reoxygenation decreased the myocardial flow heterogeneity in the chronically hypoxic heart, we speculated that its coronary vessels before reoxygenation might be near-maximum vasodilation, as in the anoxic heart, where locally coordinated vasoregulatory function does not work well. Thus, the decreased flow heterogeneity due to reoxygenation might be through the recovery of local coordination of coronary tone. As adenosine is a putative major vasoregulatory metabolite and has a preferential effect on small coronary arterioles,[20] reoxygenation might induce the constriction of small arterioles preferentially. Then the resultant decrease of coronary flow would also induce the constriction of larger resistance vessels via flow-dependent vascular responses. Elucidating the detailed mechanism underlying reoxygenation-induced vasoresponse under chronic hypoxia is, however, beyond the scope of this study.

### Implications of the data

It is difficult to describe the clinical implications of the present results. Myocardial flow heterogeneity under 8-week chronic hypoxia looks similar to the normal state despite having significant physiological and somatic changes. In another words, the integrity of the myocardial microcirculation is not compromised with chronic hypoxia, at least in resting conditions. This finding is correlated with other studies looking at the metabolism of chronically hypoxic myocardium.[21,22] Silverman et al. showed that myocardial ATP and creatine phosphate levels are maintained at rest, but they are severely depleted at cardioplegic cardiac arrest in a chronically cyanotic canine model.[21] Assessments of flow heterogeneity in hypoxic myocardium under the condition of the maximum stress, such as cardioplegic cardiac arrest or β-receptor stimulation, may be helpful for determining whether myocardial flow heterogeneity under chronic hypoxia, including its response to reoxygenation, is protective or detrimental to myocardium.

### Validation of the animal model

A rat exposed to chronic hypoxia has been established as an animal model for testing various medical and surgical interventions for cyanotic congenital heart diseases.[23,24] It has also been used as a model for primary pulmonary hypertension.[10] Chronic hypoxia induces suprasystemic RV systolic pressure as a result of high pulmonary vascular resistance and subsequent significant RV hypertrophy. The hemodynamics and physiology of this model is close to those in the moderate to severe form of tetralogy of Fallot accompanying high RV pressure and RV hypertrophy due to significant RV outflow tract obstruction. The mean $PaO_2$ of 50 mmHg and $SaO_2$ of 77% observed in the present hypoxic model are close to the values in patients undergoing elective repair for tetralogy of Fallot at 5–6 months of age.[25] This model, however, does not resemble the intracardiac mixing-type cyanotic congenital heart diseases that are usually exposed to some degree of volume overload, not

a significant pressure overload and hence no or minimal ventricular hypertrophy. In summary, this chronic model reproduces the clinical physiology of tetralogy of Fallot and its variant quite adequately, and therefore it is considered to be a reasonable animal model to investigate the collective effect of chronic cyanosis and pressure overload on myocardial blood flow heterogeneity.

### Digital radiography

Digital radiography using a molecular flow tracer is a useful method for visualizing regional myocardial flow distribution at microvascular levels. The microsphere method has been a gold standard technique to measure regional myocardial flow; however, embolization of the microsphere itself and flow biasing at vascular bifurcations makes it difficult to measure regional flow at the microvascular level.[1] Tritium-labeled DMI is an ideal molecular flow tracer; it is delivered to tissue in proportion to the local flow, nearly completely extracted during a single pass, and stably deposited at $\alpha_2$-receptors almost exclusively in capillaries. Furthermore, the path length of a b-particle emitted from tritium is short (on average 1.1 μm in a material of density).[1,4] Therefore, the radiation intensity in a small capillary tissue unit is proportional to the DMI density (i.e., the flow).[26] Stochastic and methodological errors are considered insignificant because a large number of DMI molecules are deposited in one unit (i.e., one-pixel tissue size) without vascular embolization.

### Study limitations

This study is rather descriptive, thereby documenting the myocardial flow heterogeneity in chronically hypoxic rats. Therefore, the underlying mechanism determining flow heterogeneity is beyond the scope of this study. Second, only relative flow distributions were evaluated in this study. The simultaneous measurement of the coronary blood flow and echocardiography will provide better understanding what happens in regional myocardial flow under chronic hypoxia. In addition, the postnatal exposure to hypoxia for 8 weeks may be too short to reproduce chronically hypoxic myocardium. Finally, only resting conditions were studied. Measuring the flow heterogeneity at stress (e.g., cardioplegic cardiac arrest or β-receptor stimulations) is therefore expected to provide clinically relevant information.

## Conclusions

The chronically hypoxic neonatal myocardium exhibits nearly normal regional flow heterogeneity in both the left and right ventricles. The reoxygenation of chronically hypoxic myocardium resulted in a significant decrease in regional flow heterogeneity. Elucidating the mechanism determining myocardial flow heterogeneity and its clinical implications under chronic hypoxia requires further studies under stress conditions, including evaluation of both cardiac function and coronary artery flow.

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## References

1. Matsumoto T, Kajiya F. Microheterogeneity of myocardial blood flow. Basic Res Cardiol 2001;96:547–52.
2. Deussen A. Blood flow heterogeneity in the heart. Basic Res Cardiol 1998;93:430–8.
3. King RB, Bassingthwaighte JB, Hales JR, Rowell LB. Stability of heterogeneity of myocardial blood flow in normal awake baboons. Circ Res 1985;57:285–95.
4. Austin RE Jr, Smedira NG, Squiers TM, Hoffman JI. Influence of cardiac contraction and coronary vasomotor tone on regional myocardial blood flow. Am J Physiol 1994;266: H2542–53.
5. Matsumoto T, Goto M, Tachibana H, Ogasawara Y, Tsujioka K, Kajiya F. Microheterogeneity of myocardial blood flow in rabbit hearts during normoxic and hypoxic states. Am J Physiol 1996;270:H435–41.
6. Weiss HR, Neubauer JA, Lipp JA, Sinha AK. Quantitative determination of regional oxygen consumption in the dog heart. Circ Res 1978;42:394–401.
7. Austin RE Jr, Aldea GS, Coggins DL, Flynn AE, Hoffman JI. Profound spatial heterogeneity of coronay reserve: discordance between patterns of resting and maximal myocardial blood flow Circ Res 1990;67;319–31
8. Najm HK, Wallen WJ, Belanger MP, Williams WG, Coles JG, Van Arsdell GS, et al. Does the degree of cyanosis affect myocardial adenosine triphosphate levels and function in children undergoing surgical procedures for congenital heart disease? J Thorac Cardiovasc Surg 2000;119:515–24.
9. Tajima M, Katayose D, Bessho M, Isoyama S. Acute ischaemic preconditioning and chronic hypoxia independently increase myocardial tolerance to ischaemia. Cardiovasc Res 1994;28:312–9.
10. Rabinovitch M, Gamble WJ, Miettinen OS, Reid L. Age and sex influence on pulmonary hypertension of chronic hypoxia and on recovery. Am J Physiol 1981;240:H62–72.
11. Matsumoto T, Ebata J, Tachibana H, Goto M, Kajiya F. Transmural microcirculatory blood flow distribution in right and left ventricular free walls of rabbits. Am J Physiol 1999;277:H183–91.
12. Hudlicka O, Brown M, Egginton S. Angiogenesis in skeletal and cardiac muscle. Physiol Rev 1992;72:369–417.
13. Marshall JM. Roles of adenosine and nitric oxide in skeletal muscle in acute and chronic hypoxia. Adv Exp Med Biol 2001;502:349–63.
14. DeFilippis AP, Law K, Curtin S, Eckman JR. Blood is thicker than water: the management of hyperviscosity in adults with cyanotic heart disease. Cardiol Rev 2007;15:31–4.

15. Kontras SB, Bodenbender JG, Craenen J, Hosier DM. Hyperviscosity in congenital heart disease. J Pediatr 1970;76:214–20.
16. Katayama Y, Horigome H, Murakami T, Takahashi-Igari M, Miyata D, Tanaka K. Evaluation of blood rheology in patients with cyanotic congenital heart disease using a microchannel array flow analyzer. Clin Hemorheol Microcirc 2006;35:499–508.
17. Asano T, Matsumoto T, Tachibana H, Takemoto M, Kajiya F. Myocardial microvascular perfusion after transfusion of liposome-encapsulated hemoglobin evaluated in cross-circulated rat hearts using tracer digital radiography. J Artif Organs 2004;7:145–8.
18. Vicaut E, Hou X, Decuypere L, Taccoen A, Duvelleroy M. Red blood cell aggregation and microcirculation in rat cremaster muscle. Int J Microcirc Clin Exp 1994;14:14–21.
19. Daniell HB, Bagwell EE. Effects of high oxygen on coronary flow and heart force. Am J Physiol 1968;214:1454–9.
20. Liao JC, Kuo L. Interaction between adenosine and flow-induced dilation in coronary microvascular network. Am J Physiol 1997;272:H1571–81.
21. Silverman NA, Kohler J, Levitsky S, Pavel DG, Fang RB, Feinberg H. Chronic hypoxemia depresses global ventricular function and predisposes to the depletion of high-energy phosphates during cardioplegic arrest: implications for surgical repair of cyanotic congenital heart defects. Ann Thorac Surg 1984;37:304–8.
22. Friedli B, Haenni B, Moret P, Opie LH. Myocardial metabolism in cyanotic congenital heart disease studied by arteriovenous differences of lactate, phosphate, and potassium at rest and during atrial pacing. Circulation 1977;55:647–52.
23. Corno AF, Milano G, Samaja M, Tozzi P, von Segesser LK. Chronic hypoxia: a model for cyanotic congenital heart defects. J Thorac Cardiovasc Surg 2002;124:105–12.
24. Nakanishi K, Inoue M, Sugawara E, Sano S. Ischemic and reperfusion injury of cyanotic myocardium in chronic hypoxic rat model: changes in cyanotic myocardial antioxidant system. J Thorac Cardiovasc Surg 1997;114:1088–96.
25. Van Arsdell GS, Maharaj GS, Tom J, Rao VK, Coles JG, Freedom RM, et al. What is the optimal age for repair of tetralogy of Fallot? Circulation 2000;102(suppl 3):III123–9.
26. Little SE, Link JM, Krohn KA, Bassingthwaighte JB. Myocardial extraction and retention of 2-iododesmethylimipramine: a novel flow marker. Am J Physiol 1986;250:H1060–70.

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ORIGINAL ARTICLE
Hypertension and Circulatory Control

# Medetomidine, an $\alpha_2$-Adrenergic Agonist, Activates Cardiac Vagal Nerve Through Modulation of Baroreflex Control

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*Background:* Although $\alpha_2$-adrenergic agonists have been reported to induce a vagal-dominant condition through suppression of sympathetic nerve activity, there is little direct evidence that they directly increase cardiac vagal nerve activity. Using a cardiac microdialysis technique, we investigated the effects of medetomidine, an $\alpha_2$-adrenergic agonist, on norepinephrine (NE) and acetylcholine (ACh) release from cardiac nerve endings.

*Methods and Results:* A microdialysis probe was implanted into the right atrial wall near the sinoatrial node in anesthetized rabbits and perfused with Ringer's solution containing eserine. Dialysate NE and ACh concentrations were measured using high-performance liquid chromatography. Both 10 and 100 $\mu$g/kg of intravenous medetomidine significantly decreased mean blood pressure (BP) and the dialysate NE concentration, but only 100 $\mu$g/kg of medetomidine enhanced ACh release. Combined administration of medetomidine and phenylephrine maintained mean BP at baseline level, and augmented the medetomidine-induced ACh release. When we varied the mean BP using intravenous administration of phenylephrine, treatment with medetomidine significantly steepened the slope of the regression line between mean BP and log ACh concentration.

*Conclusions:* Medetomidine increased ACh release from cardiac vagal nerve endings and augmented baroreflex control of vagal nerve activity. (*Circ J* 2012; **76:** 152–159)

Key Words: Acetylcholine; Norepinephrine; Sinoatrial node; Sympathetic nervous system; Vagus nerve

The selective $\alpha_2$-adrenergic agonist, dexmedetomidine, is widely used for sedation in intensive care units because it has a less respiratory depressive effect.[1] In addition, several benefits of dexmedetomidine that favor its use in intensive care have been reported, such as reduced opioid dosage requirement. In animal studies, Hayashi et al reported that dexmedetomidine prevented epinephrine-induced arrhythmias in halothane-anesthetized dogs.[2] This antiarrhythmic effect of $\alpha_2$-adrenergic agonists may be partly ascribed to vagal activation.[3] It has already been reported that central sympathetic inhibition by an $\alpha_2$-adrenergic agonist, guanfacine, augmented the sleep-related ultradian rhythm of parasympathetic tone in patients with chronic heart failure.[4] Although $\alpha_2$-adrenergic agonists are widely recognized as inducing a vagal-dominant condition through the suppression of sympathetic nerve, there is little direct evidence that they directly increase cardiac vagal nerve activity, because such activity has been assessed only by indirect methods, such as heart rate variability, in most studies.[5]

Vanoli et al[6] reported that vagal stimulation after an acute ischemic episode effectively prevented ventricular fibrillation in dogs. Their group also indicated that the dogs that developed ventricular fibrillation during the acute ischemic episode had a significantly lower baroreflex-mediated heart rate response,[7] suggesting the importance of the baroreflex in controlling vagal function. If an $\alpha_2$-adrenergic agonist is able to activate the cardiac vagal nerve directly or via modulation of the baroreflex function, it will provide a new therapeutic option for life-threatening arrhythmias after myocardial ischemia.

Medetomidine is a racemic mixture of 2 stereoisomers, dexmedetomidine and levomedetomidine. However, because it has already been reported that levomedetomidine has no effect on cardiovascular parameters and causes no apparent sedation or analgesia,[8] the pharmacokinetics of dexmedetomidine and racemic medetomidine are almost similar. We hypothesized that medetomidine can activate the cardiac vagal nerve

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through a central action and improve the baroreflex control of vagal nerve activity. We have established a cardiac microdialysis technique for separate monitoring of neuronal norepinephrine (NE) and acetylcholine (ACh) release to the rabbit sinoatrial (SA) node in vivo.[9–11] Using this microdialysis technique, we investigated the effects of medetomidine on cardiac autonomic nerve activities innervating the SA node.

## Methods

### Surgical Preparation

Animal care was provided in accordance with the "Guiding principles for the care and use of animals in the field of physiological sciences" published by the Physiological Society of Japan. All protocols were approved by the Animal Subject Committee of the National Cerebral and Cardiovascular Center.

In this study, 31 Japanese white rabbits weighing 2.3–3.0 kg were used. Anesthesia was initiated by an intravenous injection of pentobarbital sodium (50 mg/kg) via the marginal ear vein, and then maintained at an appropriate level by continuous intravenous infusion of $\alpha$-chloralose and urethane (16 mg·kg$^{-1}$·h$^{-1}$ and 100 mg·kg$^{-1}$·h$^{-1}$) through a catheter inserted into the femoral vein. The animals were intubated and ventilated mechanically with room air mixed with oxygen. Respiratory rate and tidal volume were set at 30 cycles/min and 15 ml/kg, respectively. Systemic arterial pressure was monitored by a catheter inserted into the femoral artery. Esophageal temperature, which was measured by a thermometer (CTM-303, Terumo, Japan), was maintained between 38°C and 39°C using a heating pad.

With the animal in lateral position, a right lateral thoracotomy was performed and the right 3rd to 5th ribs were partially resected to expose the heart. After incising the pericardium, a dialysis probe was implanted as described below. Three stainless steel electrodes were attached around the thoracotomy incision for recording body surface electrocardiogram (ECG). The heart rate was determined from the ECG using a cardiotachometer. Heparin sodium (100 IU/kg) was administered intravenously to prevent blood coagulation. At the end of the experiment, the animal was killed humanely by injecting an overdose of pentobarbital sodium. In the postmortem examination, the right atrial wall was resected en bloc with the dialysis probe. The inside of the atrial wall was observed macroscopically to confirm that the dialysis membrane was not exposed to the right atrial lumen.

### Dialysis Technique

The materials and properties of the dialysis probe have been described previously.[9–12] A dialysis fiber of semipermeable membrane (length 4 mm, outer diameter 310 $\mu$m, inner diameter 200 $\mu$m, PAN-1200, molecular weight cutoff 50,000; Asahi Chemical, Tokyo, Japan) was attached at both ends to polyethylene tubes (length 25 cm, outer diameter 500 $\mu$m, inner diameter 200 $\mu$m). A fine guiding needle (length 30 mm, outer diameter 510 $\mu$m, inner diameter 250 $\mu$m) with a stainless steel rod (length 5 mm, outer diameter 250 $\mu$m) was used for the implantation of the dialysis probe. A dialysis probe was implanted into the right atrial myocardium near the junction of the superior vena cava and the right atrium. After implantation, the dialysis probe was perfused with Ringer's solution (in mmol/L: NaCl 147, KCl 4, $CaCl_2$ 3) containing a cholinesterase inhibitor eserine (100 $\mu$mol/L), at a speed of 2 $\mu$l/min using a microinjection pump (CMA/102, Carnegie Medicin, Sweden). Experimental protocols were started 120 min after implantation of the dialysis probe. The dead space between the dialysis membrane and the sample tube was taken into account at the beginning of each dialysate sampling. In protocols 1 and 2 as described below, 8 $\mu$l of phosphate buffer (pH 3.5) was added to each sample tube before dialysate sampling, and each dialysate sampling period was set at 20 min (1 sample volume=40 $\mu$l). Half of the dialysate sample was used for ACh and the other half for NE measurements. In protocol 3, 2 $\mu$l of phosphate buffer was added to each sample tube before dialysate sampling, and each dialysate sampling period was set at 5 min (1 sample volume=10 $\mu$l). In protocol 4, 4 $\mu$l of phosphate buffer was added to each sample tube before dialysate sampling, and each dialysate sampling period was set at 10 min (1 sample volume=20 $\mu$l). Dialysate NE and ACh concentrations were analyzed separately by high-performance liquid chromatography as described previously.[12,13]

### Experimental Protocols

**Protocol 1 (n=7)** Baseline dialysate was sampled before the injection of medetomidine. Thereafter, a low dose (10 $\mu$g/kg) of medetomidine was injected intravenously via the femoral vein. After allowing 20 min for hemodynamic stabilization, dialysate was sampled for 20 min (40 $\mu$l). When the hemodynamics had recovered to the baseline level, a high dose (100 $\mu$g/kg) of medetomidine was injected intravenously and another 20-min dialysate sample was collected after hemodynamic stabilization. Finally, the vagal nerves were sectioned bilaterally at the neck and a dialysate sample was collected immediately after vagotomy. In 4 rabbits, an $\alpha_2$-adrenergic antagonist, atipamezole (2.5 mg/kg), was intravenously administered before euthanasia and hemodynamic responses were recorded.

**Protocol 2 (n=7)** To prevent possible interference of medetomidine-induced hypotension with vagal nerve activity, intravenous infusion of an $\alpha_1$-adrenergic agonist, phenylephrine, was started simultaneous to intravenous injection of medetomidine. Baseline dialysate sample was collected for 20 min before medetomidine injection. Simultaneous to intravenous injection of high-dose (100 $\mu$g/kg) medetomidine, intravenous infusion of phenylephrine was started (6.6±1.2 $\mu$g·kg$^{-1}$·min$^{-1}$) to maintain the mean blood pressure (BP) at baseline level. After hemodynamic stabilization, dialysate was sampled for 20 min. Finally, dialysate was again sampled immediately after bilateral cervical vagotomy.

**Protocol 3** To investigate the effect of medetomidine on baroreflex-induced vagal ACh release, we varied the mean BP by changing the dose of intravenous phenylephrine in both the control (n=5) and medetomidine-treated (n=7) groups. In the control group, Ringer's solution was infused intravenously at 1.0 ml·kg$^{-1}$·h$^{-1}$ throughout the experiment. In the medetomidine-treated group, medetomidine was initially injected intravenously at a dose of 60 $\mu$g/kg, and thereafter continuously infused at a dose of 60 $\mu$g·kg$^{-1}$·h$^{-1}$ or a rate of 1.0 ml·kg$^{-1}$·h$^{-1}$. After baseline dialysate sampling, mean BP was increased in a stepwise manner by altering the dose of intravenous phenylephrine (maximal dose: 32.2±5.5 $\mu$g·kg$^{-1}$·min$^{-1}$ in the control group and 18.6±2.1 $\mu$g·kg$^{-1}$·min$^{-1}$ in the medetomidine-treated group). Dialysate samples were collected for 5 min at 4–7 different mean BP levels. Relations of log ACh concentrations vs. mean BP were plotted and regression lines for each animal were calculated.

**Protocol 4 (n=5)** We investigated the peripheral effects of medetomidine on heart rate and dialysate ACh concentration under electrical stimulation of the right cervical vagal nerve. Bilateral vagal nerves were exposed through a midline cervical incision and sectioned at the neck. A pair of bipolar stainless steel electrodes was attached to the efferent side of the

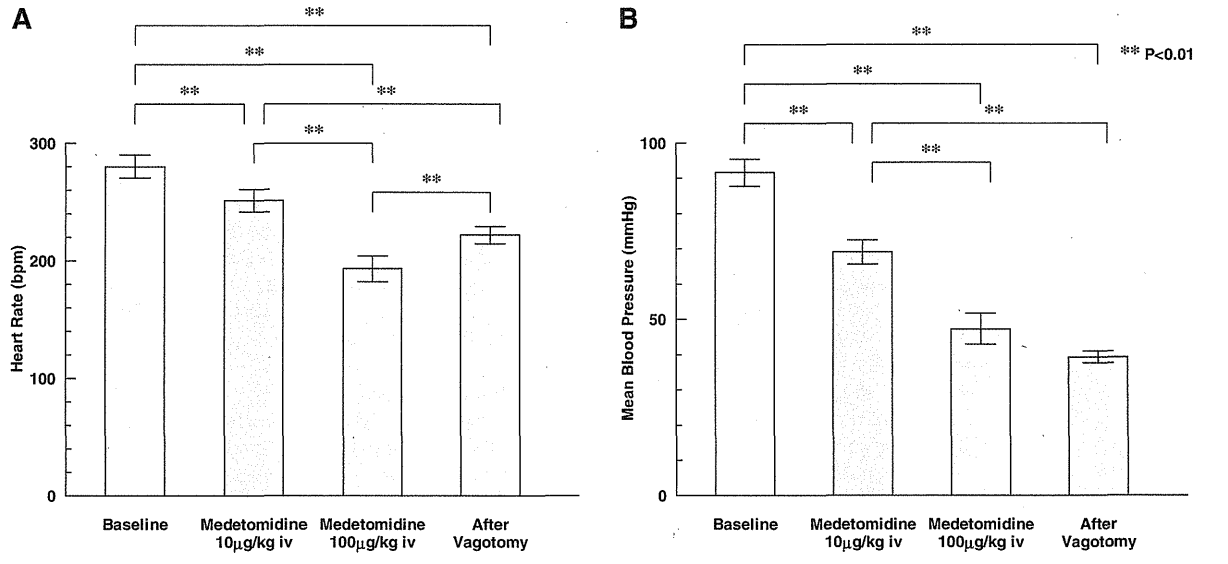


Figure 1. Intravenous administration of low and high doses of medetomidine decreased heart rate (A) and mean blood pressure (B). Medetomidine decreased heart rate in a dose-dependent manner. Vagotomy partially restored the decrease in heart rate but had no effect on mean blood pressure under administration of 100 $\mu$g/kg of medetomidine. **P<0.01.

right vagal nerve. The nerve and electrode were covered with warmed mineral oil for insulation. After the baseline dialysate sampling, the right efferent vagal nerve was stimulated at the frequency of 20 Hz by a digital stimulator (SEN-7203, Nihon Kohden, Japan). The pulse duration and amplitude of nerve stimulation were set at 1 ms and 10 V. Thereafter, a low dose (10 $\mu$g/kg) of medetomidine was injected intravenously via the femoral vein. After hemodynamic stabilization, dialysate was sampled for 10 min under the 20-Hz electrical stimulation of vagal nerve. Finally, a high dose (100 $\mu$g/kg) of medetomidine was injected intravenously and another 10-min dialysate sample was collected under the 20-Hz electrical stimulation.

### Statistical Analysis

All data are presented as mean ± standard error. Heart rate and mean BP were compared by 1-way repeated measures analysis of variance (ANOVA) followed by a Tukey's test.[14] Dialysate NE and ACh concentrations were also compared by 1-way repeated measures ANOVA followed by a Tukey's test. Comparisons of data between protocols 1 and 2 were conducted using unpaired t-test (Student's or Welch's t-test). In protocol 3, the average slopes and intercepts of the regression lines were compared using unpaired t-test. Differences were considered significant at P<0.05.

## Results

### Protocol 1

Intravenous injection of medetomidine significantly decreased heart rate (Figure 1A) and mean BP (Figure 1B) in a dose-dependent manner (280±10 beats/min and 92±4 mmHg, respectively, at baseline; 251±10 beats/min and 69±3 mmHg at 10 $\mu$g/kg; and 193±11 beats/min and 47±4 mmHg at 100 $\mu$g/kg, P<0.01 for all comparisons). Vagotomy increased heart rate to 222±7 beats/min but did not affect mean BP (Figures 1A,B).

Low-dose medetomidine significantly decreased dialysate NE concentration (Figure 2A) from 0.72±0.06 to 0.59±0.04 nmol/L (P<0.01) but did not affect dialysate ACh concentration (Figure 2B) compared with baseline. High-dose medetomidine also decreased dialysate NE concentration (to 0.52±0.05 nmol/L) similar to low-dose medetomidine (Figure 2A) and significantly increased dialysate ACh concentration from 7.2±1.3 nmol/L at baseline to 12.1±1.6 nmol/L (P<0.01, Figure 2B). Dialysate NE concentration was not changed by vagotomy, whereas dialysate ACh concentration recovered to the baseline level immediately after vagotomy (Figures 2A,B).

In 4 rabbits treated with atipamezole, heart rate and mean BP recovered to the baseline levels immediately after the injection (276±18 beats/min and 88±6 mmHg, respectively, at baseline; and 280±11 beats/min and 83±6 mmHg after the injection).

### Protocol 2

Intravenous injection of high-dose medetomidine combined with phenylephrine decreased heart rate (Figure 3A) and the decrease was significantly greater than that observed in protocol 1 (140±9 vs. 193±11 beats/min, P<0.01), while mean BP was maintained at the same level as baseline (Figure 3B). Medetomidine combined with phenylephrine decreased dialysate NE concentration from 0.85±0.09 at baseline to 0.68±0.10 nmol/L (Figure 4A), and the decrease was not significantly different from that of medetomidine alone (protocol 1). However, medetomidine combined with phenylephrine increased dialysate ACh concentration (Figure 4B) to a significantly and markedly higher level than that observed in protocol 1 (26.8±5.4 vs. 12.1±1.6 nmol/L, P<0.05). Dialysate ACh concentration recovered to the baseline level immediately after vagotomy.

### Protocol 3

The change in mean BP by phenylephrine administration affected dialysate ACh concentration only slightly in the control group (Figure 5A), whereas the elevation of mean BP

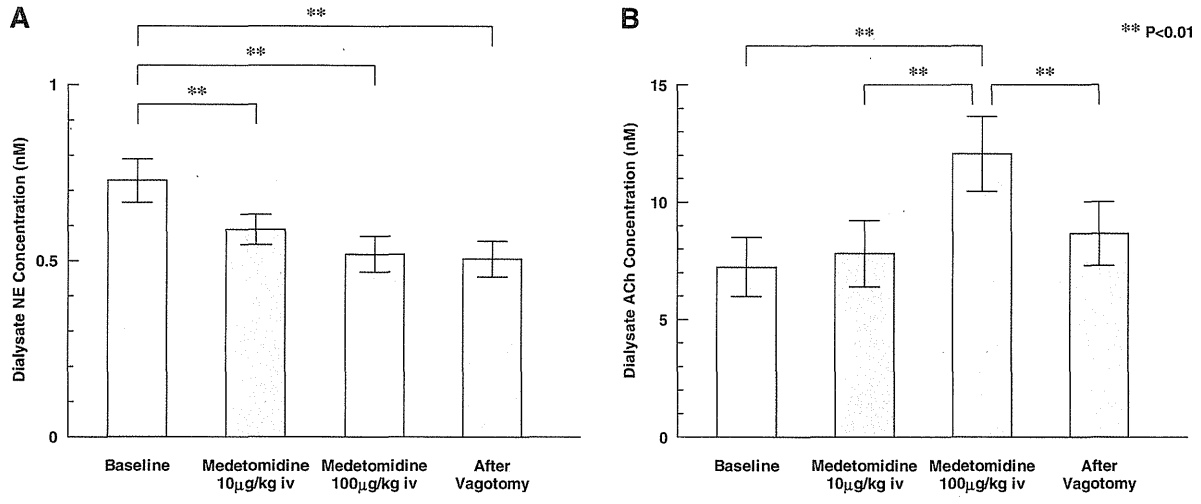


**Figure 2.** Effects of intravenous administration of low and high doses of medetomidine on dialysate NE (A) and ACh concentrations (B). Medetomidine at 10 and 100 μg/kg similarly decreased dialysate NE concentration (A). Medetomidine at 100 μg/kg significantly increased dialysate ACh concentration, but 10 μg/kg had no effect (B). Vagotomy suppressed the increase in dialysate ACh concentration. **P<0.01. ACh, acetylcholine; NE, norepinephrine.

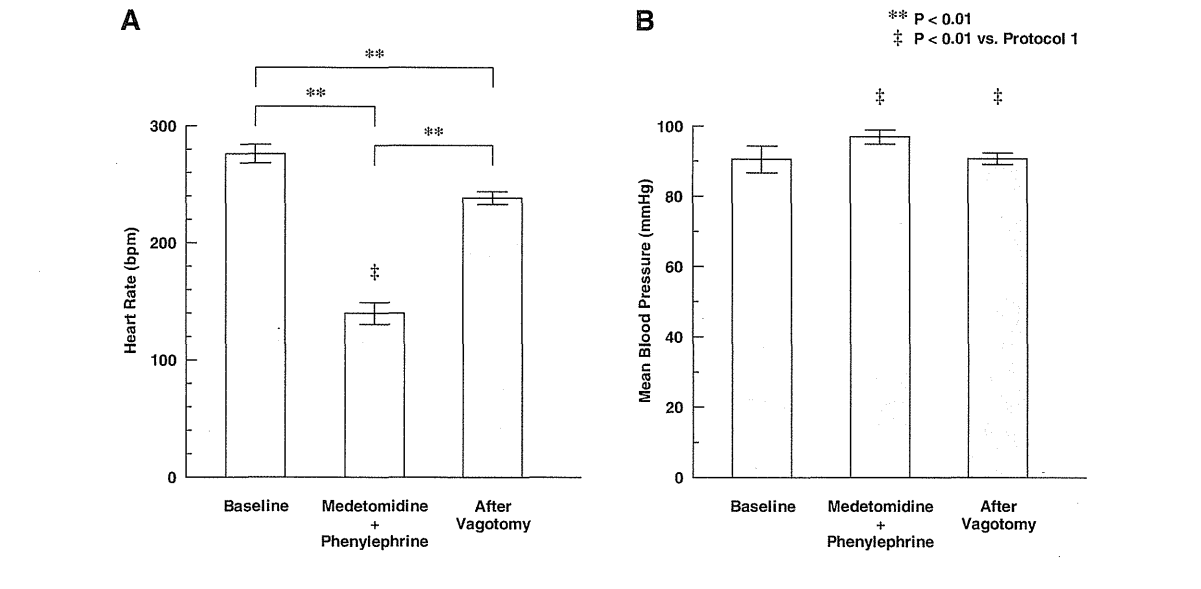


**Figure 3.** Effects of simultaneous intravenous administration of medetomidine and phenylephrine on heart rate (A) and mean blood pressure (B). Medetomidine combined with phenylephrine significantly decreased heart rate (A), and the decrease was significantly greater compared with medetomidine alone (protocol 1: Figure 1A). Medetomidine combined with phenylephrine maintained mean blood pressure (B) at the baseline level. Vagotomy partially restored the decrease in heart rate but had no effect on mean blood pressure. **P<0.01; ‡P<0.01 vs. medetomidine alone (protocol 1: Figure 1A).

markedly increased dialysate ACh concentration in the medetomidine-treated group (Figure 5B). The average slopes of the regression lines between mean BP and log dialysate ACh concentration were 0.0018±0.0004 in the control and 0.0062±0.0006 in the medetomidine-treated group. The slope was significantly steeper in the medetomidine-treated group than that in the control (P<0.01). However, the intercept did not differ significantly between the control (0.59±0.05) and medetomidine-treated (0.68±0.07) groups.

## Protocol 4

The 20-Hz electrical stimulation of the right vagal nerve significantly decreased heart rate from 271±11 beats/min at the baseline to 112±6 beats/min and increased dialysate ACh concentration from 7.1±0.9 nmol/L at the baseline to 38.5±7.2 nmol/L (P<0.01). However, both 10 and 100 μg/kg of me-

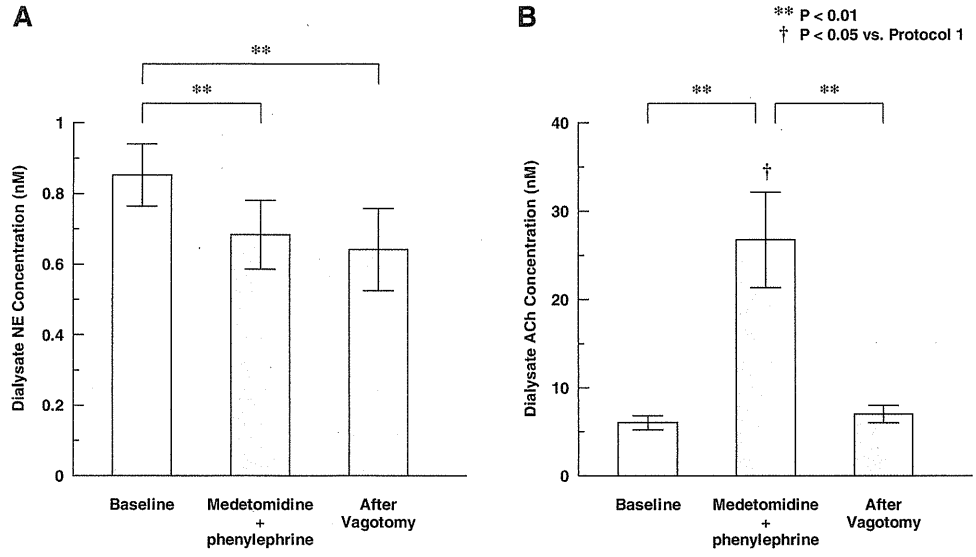


**Figure 4.** Effects of simultaneous intravenous administration of medetomidine and phenylephrine on dialysate NE (A) and ACh concentrations (B). Medetomidine combined with phenylephrine decreased dialysate NE concentration (A), and the decrease was similar to medetomidine alone (protocol 1: Figure 2A). Medetomidine combined with phenylephrine caused a marked increase in dialysate ACh concentration (B) and the increase was significantly greater than medetomidine alone (protocol 1: Figure 2B). Vagotomy suppressed the increase in dialysate ACh concentration. **P<0.01; †P<0.05 vs. medetomidine alone (protocol 1: Figure 2B). ACh, acetylcholine; NE, norepinephrine.

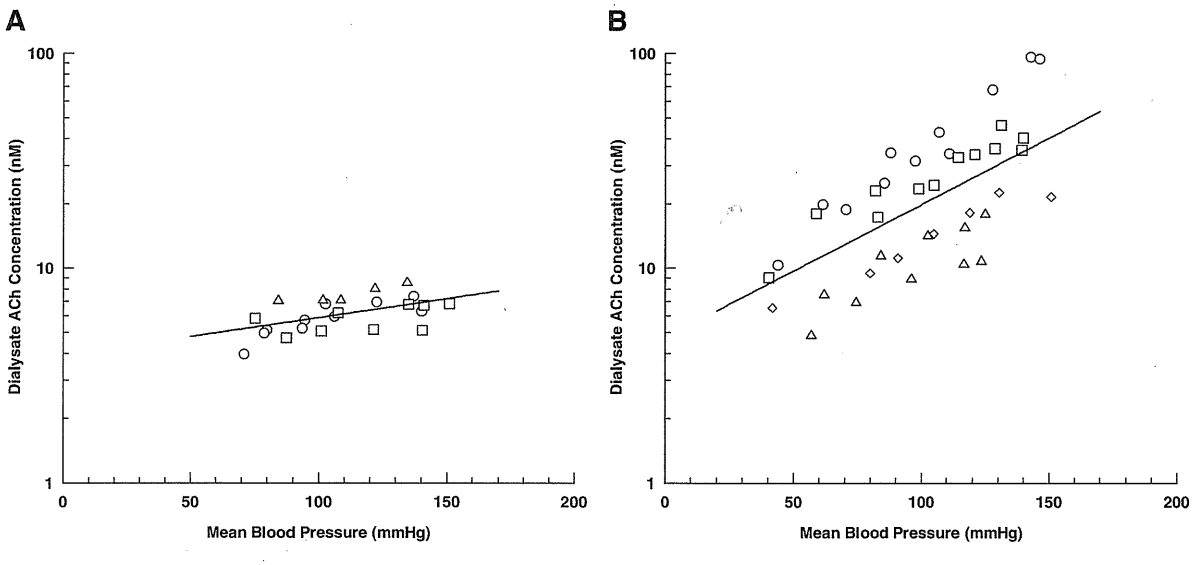


**Figure 5.** Regression lines of dialysate ACh concentration vs. MBP in control group (A) and medetomidine-treated group (B). In the control group (n=5), the increase in MBP had little effect on dialysate ACh concentration (A). In the medetomidine-treated group (n=7), dialysate ACh concentration was elevated with increase in MBP (B). Average regression line for control group: log[ACh]= 0.0018×MBP+0.59. Average regression line for medetomidine-treated group: log[ACh]=0.0062×MBP+0.68. Each symbol represents the data of 1 animal. ACh, acetylcholine; MBP, mean blood pressure.

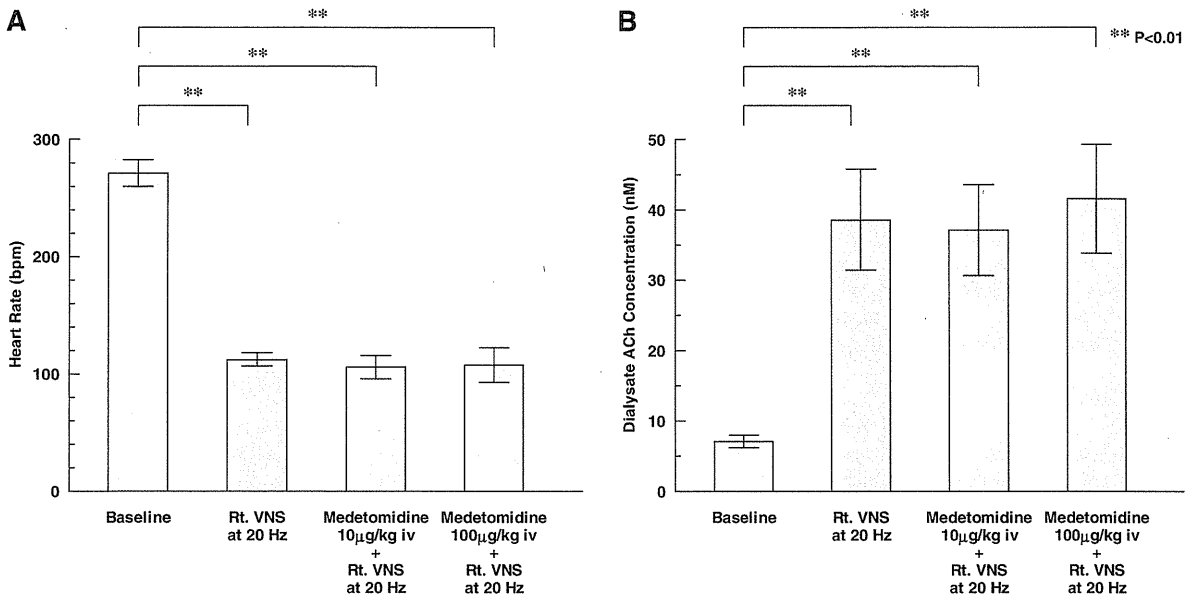


**Figure 6.** Peripheral effects of medetomidine on heart rate (A) and dialysate acetylcholine (ACh) concentration (B) under electrical stimulation of the right cervical vagal nerve. The 20-Hz electrical stimulation of the right cervical vagal nerve significantly decreased heart rate and increased dialysate ACh concentration. However, medetomidine did not affect the heart rate and dialysate ACh concentration under electrical vagal nerve stimulation (VNS).

detomidine did not affect heart rate and dialysate ACh concentration under the electrical stimulation (106±9.9 beats/min and 37.1±6.1 nmol/L at 10 $\mu$g/kg, 108±15 beats/min and 41.6±7.7 nmol/L at 100 $\mu$g/kg).

## Discussion

We have elucidated the effects of medetomidine on cardiac sympathetic and vagal nerve activities simultaneously using cardiac microdialysis technique. Intravenous administration of 10 $\mu$g/kg of medetomidine significantly decreased sympathetic NE release to the SA node, while intravenous administration of 100 $\mu$g/kg of medetomidine significantly increased vagal ACh release to the SA node in addition to sympathetic suppression.

### $\alpha_2$-Adrenergic Agonist and Cardiac Sympathetic Nerve Activity

It is well-documented that $\alpha_2$-adrenergic agonist suppresses sympathetic nerve activity.[15] Oku et al reported that dexmedetomidine suppressed renal sympathetic nerve discharge in baroreceptor-denervated rabbits.[16] In the present study, low-dose medetomidine decreased heart rate and mean BP through inhibiting sympathetic nerve activity, without affecting cardiac vagal nerve activity. High-dose medetomidine also suppressed NE release to the same level as low-dose medetomidine.

Several mechanisms may be involved in the sympathoinhibitory effect of $\alpha_2$-adrenergic agonist. The rostral ventrolateral medulla has been reported to serve as an important site in mediating the hypotensive and sedative effects of $\alpha_2$-adrenergic agonist.[17] McCallum et al reported that the central sympathoinhibitory effects of $\alpha_2$-adrenoceptor stimulation are augmented by peripheral inhibition of ganglionic transmission.[18] The results obtained from protocol 1 indicate that low-dose medetomidine may induce a vagal-dominant condition through suppression of the cardiac sympathetic nerve without direct activation of the cardiac vagal nerve.

### $\alpha_2$-Adrenergic Agonist and Cardiac Vagal Nerve Activity

Kamibayashi et al reported that the vagus nerve played an important role in the antidysrhythmic effect of dexmedetomidine.[3] However, because it is difficult to selectively monitor cardiac vagal nerve activity, there is little direct evidence that $\alpha_2$-adrenergic agonists can directly increase cardiac vagal nerve activity. In the present study, high-dose medetomidine significantly decreased heart rate and mean BP compared with low-dose medetomidine in protocol 1, and analyses of NE and ACh release by microdialysis technique proved that these decreases in heart rate and mean BP were associated with an increase in vagal ACh release to the heart. Histocytological studies demonstrated the presence of $\alpha_2$-adrenergic receptors in the vagal dorsal motor nucleus and nucleus tractus solitarius.[19] Therefore, it is possible that $\alpha_2$-adrenergic agonists directly activate the cardiac vagal nerve. It is also possible that intravenous medetomidine also modulates vagal ACh release through ganglionic transmission and the direct action to nerve endings. In protocol 4, however, medetomidine did not affect heart rate or the dialysate ACh concentration under electrical stimulation of the right efferent vagal nerve. Thus, in our experimental setting the peripheral effects of medetomidine on cardiac vagal nerve activity may be small compared with its central effects.

To exclude the possibility that medetomidine-induced hypotension affects local ACh concentrations, the mean BP was maintained constant by co-administration of phenylephrine in protocol 2. High-dose medetomidine combined with phenylephrine enhanced the decrease in heart rate and the increase in dialysate ACh concentration without medetomi-

dine-induced hypotension, indicating that hypotension occurring in protocol 1 had actually reduced ACh release in response to high-dose medetomidine. The results also suggest an interaction between baroreflex-induced and medetomidine-induced vagal nerve activation, which was extensively examined in protocol 3. In protocol 3, medetomidine steepened the slope of the regression line between mean BP and log dialysate ACh concentration, without affecting the intercept. In other words, medetomidine enhanced the baroreflex-induced ACh release from cardiac vagal nerve endings. Because the central pathway of baroreflex includes the vagal dorsal motor nucleus and nucleus tractus solitarius, in which $\alpha_2$-adrenergic receptors have been demonstrated,[19] medetomidine may act on this pathway and modulate baroreflex-induced ACh release.

### Clinical Implication

The selective $\alpha_2$-adrenergic agonist, dexmedetomidine, is widely used for sedation in intensive care units. Bradycardia and hypotension are known to be unfavorable events during dexmedetomidine sedation.[20] Some cases of dexmedetomidine-induced atrioventricular block followed by cardiac arrest have been reported.[21,22] This critical complication may be associated with direct vagal activation by the $\alpha_2$-adrenergic agonist. Compared with our previous results of electrical cervical vagal nerve stimulation in rabbits,[9] intravenous administration of 100 $\mu$g/kg of medetomidine had an effect equivalent to electrical vagal stimulation at 10 Hz. Furthermore, when the mean BP was maintained constant using phenylephrine, medetomidine had a stronger effect on cardiac vagal nerve activity, which is similar to 20-Hz electrical vagal stimulation, and this magnitude may sometimes cause atrioventricular block or sinus arrest.

Notwithstanding these adverse effects, vagal activation has several favorable cardioprotective effects. Our study proved that medetomidine, a selective $\alpha_2$-adrenergic agonist, is a strong activator of cardiac vagal nerve. Vanoli et al[6] reported that vagal stimulation after acute ischemia can prevent ventricular fibrillation. Ando et al reported that efferent vagal nerve stimulation prevented ischemia-induced arrhythmias by preserving connexin 43 protein.[23] Our results suggest that vagal activation in addition to sympathetic suppression probably contributes to the antiarrhythmic effect of medetomidine.

Because inhibition of the sympathetic nerve system has been the cornerstone of drug therapy for heart failure,[24] a selective $\alpha_2$-adrenergic agonist may be a potential therapeutic option for heart failure. Recent studies have shown that electrical vagal nerve stimulation also improves the outcomes in patients with heart failure.[25] Electrical stimulation of carotid baroreceptor has recently been reported to be a therapeutic option for heart failure. Sabbah et al reported that chronic electrical stimulation of the carotid sinus baroreflex improved left ventricular function and promoted reversal of ventricular remodeling in dogs with advanced heart failure.[26] Our study demonstrated that medetomidine modulates baroreflex control to enhance vagal nerve activity, which may also induce further cardioprotective effects.

### Study Limitations

First, ACh is degraded by ACh esterase immediately after release. Therefore, detection of in vivo ACh release requires the addition of eserine, a specific ACh esterase inhibitor, into the perfusate. The presence of eserine around the semipermeable membrane might have affected ACh release in the vicinity of the semipermeable membrane. Eserine could have activated regulatory pathways such as autoinhibition of ACh release via muscarinic receptors.

Second, medetomidine is a chiral imidazole derivative. Thus, imidazoline receptors may also be involved in the cardiac vagal activation by medetomidine. Further investigation is necessary to clarify the influence of imidazoline receptors on cardiac vagal nerve activity. However, because an $\alpha_2$-adrenergic antagonist, atipamezole, abolished the hemodynamic responses to medetomidine, we think that the cardiovascular effects of medetomidine are mainly related to the direct action of $\alpha_2$-adrenergic receptors.

Third, the interactive effects between sympathetic and vagal nerve endings remain uncertain in the present study. Thus, we need further investigations including the open-loop approach where baroreceptor input pressure is strictly controlled.

## Conclusion

A selective $\alpha_2$-adrenergic agonist, medetomidine, directly activates cardiac vagal nerve and enhances the baroreflex control of vagal nerve activity. Medetomidine may be a therapeutic option for life-threatening arrhythmia or heart failure if the adverse effects are properly managed.


## Acknowledgments

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## References


1. Hsu YW, Cortinez LI, Robertson KM, Keifer JC, Sum-Ping ST, Moretti EW, et al. Dexmedetomidine pharmacodynamics: Part I: Crossover comparison of the respiratory effects of dexmedetomidine and remifentanil in healthy volunteers. *Anesthesiology* 2004; **101:** 1066–1076.
2. Hayashi Y, Sumikawa K, Maze M, Yamatodani A, Kamibayashi T, Kuro M, et al. Dexmedetomidine prevents epinephrine-induced arrhythmias through stimulation of central alpha 2 adrenoceptors in halothane-anesthetized dogs. *Anesthesiology* 1991; **75:** 113–117.
3. Kamibayashi T, Hayashi Y, Mammoto T, Yamatodani A, Sumikawa K, Yoshiya I. Role of the vagus nerve in the antidysrhythmic effect of dexmedetomidine on halothane/epinephrine dysrhythmias in dogs. *Anesthesiology* 1995; **83:** 992–999.
4. Yamazaki T, Asanoi H, Ueno H, Yamada K, Takagawa J, Kameyama T, et al. Central sympathetic inhibition augments sleep-related ultradian rhythm of parasympathetic tone in patients with chronic heart failure. *Circ J* 2005; **69:** 1052–1056.
5. Lombardi F, Sandrone G, Pernpruner S, Sala R, Garimoldi M, Cerutti S, et al. Heart rate variability as an index of sympathovagal interaction after acute myocardial infarction. *Am J Cardiol* 1987; **60:** 1239–1245.
6. Vanoli E, De Ferrari GM, Stramba-Badiale M, Hull SS Jr, Foreman RD, Schwartz PJ. Vagal stimulation and prevention of sudden death in conscious dogs with a healed myocardial infarction. *Circ Res* 1991; **68:** 1471–1481.
7. Schwartz PJ, La Rovere MT, Vanoli E. Autonomic nervous system and sudden cardiac death: Experimental basis and clinical observations for post-myocardial infarction risk stratification. *Circulation* 1992; **85:** I77–I91.
8. Kuusela E, Raekallio M, Anttila M, Falck I, Mölsä S, Vainio O. Clinical effects and pharmacokinetics of medetomidine and its enantiomers in dogs. *J Vet Pharmacol Ther* 2000; **23:** 15–20.
9. Shimizu S, Akiyama T, Kawada T, Shishido T, Yamazaki T, Kamiya A, et al. In vivo direct monitoring of vagal acetylcholine release to the sinoatrial node. *Auton Neurosci* 2009; **148:** 44–49.
10. Shimizu S, Akiyama T, Kawada T, Shishido T, Mizuno M, Kamiya A, et al. In vivo direct monitoring of interstitial norepinephrine levels at the sinoatrial node. *Auton Neurosci* 2010; **152:** 115–118.
11. Shimizu S, Akiyama T, Kawada T, Sonobe T, Kamiya A, Shishido T, et al. Centrally administered ghrelin activates cardiac vagal nerve

in anesthetized rabbits. *Auton Neurosci* 2011; **162:** 60–65.
12. Akiyama T, Yamazaki T, Ninomiya I. In vivo monitoring of myocardial interstitial norepinephrine by dialysis technique. *Am J Physiol* 1991; **261:** H1643–H1647.
13. Akiyama T, Yamazaki T, Ninomiya I: In vivo detection of endogenous acetylcholine release in cat ventricles. *Am J Physiol* 1994; **266:** H854–H860.
14. Glantz SA. Primer of biostatistics, 6th edn. New York: McGraw-Hill, 2005.
15. Heusch G, Schipke J, Thämer V. Clonidine prevents the sympathetic initiation and aggravation of poststenotic myocardial ischemia. *J Cardiovasc Pharmacol* 1985; **7:** 1176–1182.
16. Oku S, Benson KT, Hirakawa M, Goto H. Renal sympathetic nerve activity after dexmedetomidine in nerve-intact and baroreceptor-denervated rabbits. *Anesth Analg* 1996; **83:** 477–481.
17. Yamazato M, Sakima A, Nakazato J, Sesoko S, Muratani H, Fukiyama K. Hypotensive and sedative effects of clonidine injected into the rostral ventrolateral medulla of conscious rats. *Am J Physiol Regul Integr Comp Physiol* 2001; **281:** R1868–R1876.
18. McCallum JB, Boban N, Hogan Q, Schmeling WT, Kampine JP, Bosnjak ZJ. The mechanism of alpha2-adrenergic inhibition of sympathetic ganglionic transmission. *Anesth Analg* 1998; **87:** 503–510.
19. Robertson HA, Leslie RA. Noradrenergic alpha 2 binding sites in vagal dorsal motor nucleus and nucleus tractus solitarius: Autoradiographic localization. *Can J Physiol Pharmacol* 1985; **63:** 1190–1194.
20. Candiotti KA, Bergese SD, Bokesch PM, Feldman MA, Wisemandle W, Bekker AY; MAC Study Group. Monitored anesthesia care with dexmedetomidine: A prospective, randomized, double-blind, multicenter trial. *Anesth Analg* 2010; **110:** 47–56.
21. Nagasaka Y, Machino A, Fujikake K, Kawamoto E, Wakamatsu M. Cardiac arrest induced by dexmedetomidine. *Masui* 2009; **58:** 987–989.
22. Ingersoll-Weng E, Manecke GR Jr, Thistlethwaite PA. Dexmedetomidine and cardiac arrest. *Anesthesiology* 2004; **100:** 738–739.
23. Ando M, Katare RG, Kakinuma Y, Zhang D, Yamasaki F, Muramoto K, et al. Efferent vagal nerve stimulation protects heart against ischemia-induced arrhythmias by preserving connexin43 protein. *Circulation* 2005; **112:** 164–170.
24. Sata Y, Krum H. The future of pharmacological therapy for heart failure. *Circ J* 2010; **74:** 809–817.
25. Schwartz PJ. Vagal stimulation for heart diseases: From animals to men: An example of translational cardiology. *Circ J* 2010; **75:** 20–27.
26. Sabbah HN, Gupta RC, Imai M, Irwin ED, Rastogi S, Rossing MA, et al. Chronic electrical stimulation of the carotid sinus baroreflex improves left ventricular function and promotes reversal of ventricular remodeling in dogs with advanced heart failure. *Circ Heart Fail* 2011; **4:** 65–70.

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ORIGINAL ARTICLE
Pulmonary Circulation

# Safety and Efficacy of Epoprostenol Therapy in Pulmonary Veno-Occlusive Disease and Pulmonary Capillary Hemangiomatosis

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*Background:* Pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH) are rare causes of pulmonary hypertension. There is no proven medical therapy to treat these diseases, and lung transplantation is thought to be the only cure. Administration of vasodilators including epoprostenol sometimes causes massive pulmonary edema and could be fatal in these patients.

*Methods and Results:* Eight patients were treated with epoprostenol for 387.3±116.3 days (range, 102–1,063 days), who were finally diagnosed with PVOD or PCH by pathological examination. The maximum dose of epoprostenol given was 55.3±10.7 ng·kg$^{-1}$·min$^{-1}$ (range, 21.0–110.5 ng·kg$^{-1}$·min$^{-1}$). With careful management, epoprostenol therapy significantly improved the 6-min walk distance (97.5±39.2 to 329.4±34.6 m, P<0.001) and plasma brain natriuretic peptide levels (381.3±136.8 to 55.2±14.4 pg/ml, P<0.05). The cardiac index significantly increased from 2.1±0.1 to 2.9±0.3 L·min$^{-1}$·m$^{-2}$ (P<0.05). However, pulmonary artery pressure and pulmonary vascular resistance were not significantly reduced. For 4 patients, epoprostenol therapy acted as a bridge to lung transplantation. For the other patients who had no chance to undergo lung transplantation, epoprostenol therapy was applied for 528.0±216.6 days and the maximum dose was 63.9±19.0 ng·kg$^{-1}$·min$^{-1}$.

*Conclusions:* This study data suggest that cautious application of epoprostenol can be considered as a therapeutic option in patients with PVOD and PCH. (*Circ J* 2012; **76:** 1729–1736)

Key Words: Epoprostenol; Pulmonary capillary hemangiomatosis; Pulmonary hypertension; Pulmonary veno-occlusive disease

Pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH) are rare causes of pulmonary hypertension, and their categories have been changed at every World Symposium on Pulmonary Hypertension.[1,2] The latest clinical classification of pulmonary hypertension categorized these diseases as Group 1' considering the similarity of risk factors and the genetic mutations in idiopathic pulmonary arterial hypertension (IPAH).[3,4] Continuous intravenous infusion of epoprostenol decreases pulmonary vascular resistance and improves the prognosis of IPAH,[5,6] and it has become a standard therapy for IPAH. However, the indication of epoprostenol for other subgroups of pulmonary hypertension including PVOD and PCH is controversial. A few patients with PVOD have been reported to show amelioration by application of epoprostenol.[7,8] In contrast, other reports have warned that epoprostenol precipitates severe pulmonary edema in patients with PVOD or PCH,[9,10] which never occurs in patients with IPAH. This is why epoprostenol is not widely accepted as a standard therapy for PVOD and PCH.

Montani et al reported the possible efficacy of epoprostenol for PVOD as a bridge to lung transplantation.[11] They successfully treated 12 patients (10 patients with PVOD proven by pathological studies and 2 patients with a clinical diagnosis of PVOD) for 210 days with a maximal dose of 13 ng·kg$^{-1}$·min$^{-1}$ of epoprostenol. This was the first report to show the clinical application of epoprostenol therapy in a series of patients with PVOD. However, no reports have described the successful

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Table 1. Baseline Data, Histological Diagnosis and Outcome

| Patient no. | Age (years) | Sex | WHO FC | Mean PAP (mmHg) | %DLco (%) | Histological diagnosis | Outcome |
|---|---|---|---|---|---|---|---|
| 1 | 42 | M | III | 39 | 24 | PVOD | Death |
| 2 | 26 | M | IV | 60 | 31 | PVOD | Death |
| 3 | 29 | M | IV | 114 | NA | PVOD | Death |
| 4 | 11 | M | IV | 52 | 64 | PCH | Death |
| 5 | 25 | F | IV | 55 | 36 | PCH | LDLLT |
| 6 | 28 | F | III | 65 | 81 | PVOD | LDLLT |
| 7 | 16 | F | III | 63 | 61 | PVOD | LDLLT |
| 8 | 32 | F | III | 44 | 23 | PVOD | LDLLT |

Age, age at diagnosis; WHO FC, World Health Organization classification of functional status of patients with pulmonary hypertension; PAP, pulmonary artery pressure; %DLco, diffusion capacity of the lung for carbon monoxide expressed as % predicted; M, male; F, female; PVOD, pulmonary veno-occlusive disease; PCH, pulmonary capillary hemangiomatosis; LDLLT, living-donor lobar lung transplantation.

application of epoprostenol for PCH. We report on 8 patients (6 patients with PVOD and 2 with PCH) whose diagnoses were confirmed by pathological examination, and who were treated with a higher dose of epoprostenol and for a longer period than previously reported. With great caution, epoprostenol was safely applied and improved the clinical status in all patients. Careful application of long-term epoprostenol therapy appears to be a safe option and results in a favorable therapeutic outcome in patients with PVOD and PCH.

## Methods

We treated patients with pulmonary hypertension with epoprostenol at 2 institutions (Okayama University Hospital and National Hospital Organization Okayama Medical Center, Okayama, Japan) between April 1999 and April 2010. Diagnosis of pulmonary hypertension was made according to a standard diagnostic algorithm including physical examination, chest radiograph, blood tests including screening for the cause of secondary pulmonary hypertension, pulmonary function testing, transthoracic Doppler echocardiography, and right heart catheterization.[12]

Eight patients had the clinical diagnosis of pulmonary hypertension, which was finally determined to be PVOD or PCH, in this study period. We performed a standardized chart review from the medical records to extract clinical data from them retrospectively. We compared clinical, hemodynamic, and radiographic data before and after application of epoprostenol. Data after epoprostenol treatment were obtained at the time when patients achieved the best values for the cardiac index by right heart catheterization.

Seven patients underwent pulmonary function tests when first admitted to our hospital. Vital capacity and forced expiratory volume at 1 s were calculated by using standard formulas. Diffusion capacity of the lung for carbon monoxide (DLco) was measured by the single-breath method and expressed as %DLco (% predicted). Cardiac catheterization was routinely performed at baseline before starting epoprostenol therapy and then repeatedly after starting epoprostenol therapy according to the patients' condition. Chest radiographs were obtained from all patients at the initial visit and were repeatedly taken according to their status. All patients underwent high-resolution computed tomography (CT) of the chest to define coexisting conditions, including pulmonary venous congestion, pulmonary arterial enlargement, atelectasis, or pleural effusion.

### Titration of Epoprostenol Therapy

Epoprostenol therapy was initiated at a dose of 0.25–0.5 $ng \cdot kg^{-1} \cdot min^{-1}$, and the dose was gradually titrated upward in increments of 0.5–1.0 $ng \cdot kg^{-1} \cdot min^{-1}$, based on adverse effects and tolerance. When the cardiac index was below 2.0 $L \cdot min^{-1} \cdot m^{-2}$, continuous intravenous catecholamines were added to epoprostenol therapy. On adjusting the dose of epoprostenol, we paid careful attention to hypotension and signs of deterioration of heart failure and pulmonary edema. When the patients' chest radiographs showed deterioration, we stopped increasing the dose of epoprostenol and added diuretics or intravenous infusion of catecholamines, depending on the severity of pulmonary edema. After improvement, titration of the dose of epoprostenol was resumed.

### Pathological Examination

No open or thoracoscopic lung biopsy was performed in any of the patients, because all patients were severely ill and they were considered intolerable to a lung biopsy. Lung specimens were obtained by living-donor lobar lung transplantation (LDLLT) or autopsy. Lung tissue was fixed in 10% formalin. Histological sections were stained with hematoxylin and eosin stain and elastica-Masson's trichrome stain.

### Statistical Analysis

Results are reported as mean±standard error of the mean. Differences between groups in variables measured at baseline and after epoprostenol therapy were tested by the paired t-test. Differences were considered statistically significant at a P value of $<0.05$.

## Results

### Baseline Data, Pathological Findings and Outcome

Eight patients undergoing epoprostenol therapy had the histological diagnosis of PVOD or PCH (Table 1). The patients included 4 males and 4 females with a mean age of 26.0±3.4 years at the time of diagnosis of pulmonary hypertension. At baseline, 4 patients with PVOD were in the World Health Organization (WHO) functional class III and the other 4 patients (PVOD, n=2; PCH, n=2) were in the functional class IV. All patients showed a high mean pulmonary artery pressure (PAP) and 4 patients showed a marked decrease in %DLco as low as below 40%.

Two patients (patients 4 and 5) were finally diagnosed with PCH and the other cases were diagnosed with PVOD. Representative histology is shown in Figure 1. In all cases, foci of

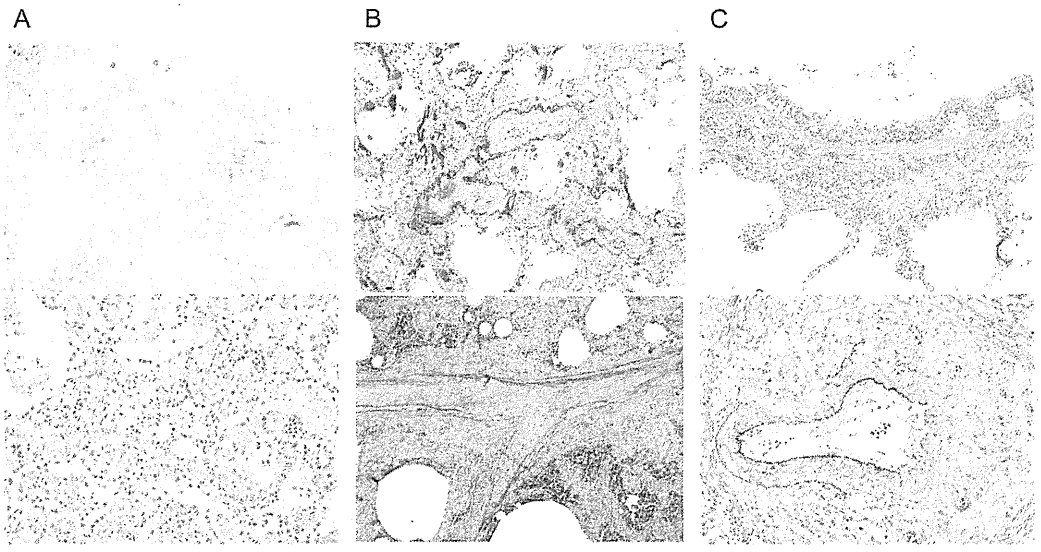


**Figure 1.** Pathological findings of lung specimens. (A) Specimens of pulmonary veno-occlusive disease (PVOD) show centrilobular congestion at low magnification (Upper panel) and characteristic alveolar capillaries at a higher magnification (Lower panel). These foci are seen in both PVOD and pulmonary capillary hemangiomatosis (PCH) (hematoxylin and eosin stain). (B) Venous vessel walls are thickened by intimal fibrous proliferation. Markedly stenosed (Upper panel) and completely obliterated (Lower panel) veins can be seen in PVOD (elastica-Masson's trichrome stain). (C) Proliferating capillaries are shown in the walls of bronchi (Upper panel) and arteries (Lower panel) in PCH (elastica-Masson's trichrome stain).

**Table 2. Clinical and Hemodynamic Data Before and After Epoprostenol Therapy**

| | Baseline | After epoprostenol therapy | P value |
|---|---|---|---|
| **WHO FC (n)** | | | |
| II | 0 | 5 | |
| III | 4 | 3 | |
| IV | 4 | 0 | |
| **6MWD (m)** | 97.5±39.2 | 329.4±34.6 | <0.001 |
| **BNP (pg/ml)** | 381.3±136.8 | 55.2±14.4 | <0.05 |
| **Hemodynamics** | | | |
| Systolic PAP (mmHg) | 89.4±11.0 | 90.9±4.9 | NS |
| Diastolic PAP (mmHg) | 44.1±7.2 | 43.4±4.0 | NS |
| Mean PAP (mmHg) | 61.5±8.1 | 61.5±3.9 | NS |
| PCWP (mmHg) | 7.0±1.3 | 11.8±3.6 | NS |
| RAP (mmHg) | 6.9±2.2 | 7.6±1.5 | NS |
| $SvO_2$ (%) | 59.6±5.3 | 64.9±4.8 | NS |
| CI ($L \cdot min^{-1} \cdot m^{-2}$) | 2.1±0.1 | 2.9±0.3 | <0.05 |
| PVR ($dyne \cdot s \cdot cm^{-5}$) | 1,449.3±194.9 | 1,096.3±199.5 | NS |
| **Epoprostenol therapy** | | | |
| Duration (days) | | 164.1±79.7 | |
| Dose ($ng \cdot kg^{-1} \cdot min^{-1}$) | | 24.4±5.6 | |
| **Associated therapy (n)** | | | |
| Anticoagulation | 8 | 6 | |
| Digitalis | 4 | 3 | |
| Bosentan | 2 | 2 | |
| Sildenafil | 2 | 2 | |

After epoprostenol therapy, at the time when patients achieved the best values for cardiac index; 6MWD, 6-min walk distance; BNP, plasma concentrations of brain natriuretic peptide; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; $SvO_2$, mixed venous oxygen saturation; CI, cardiac index; PVR, pulmonary vascular resistance; duration, time from initiation of epoprostenol; NS, not significant; dose, dose of epoprostenol. All other abbreviations are as per Table 1.

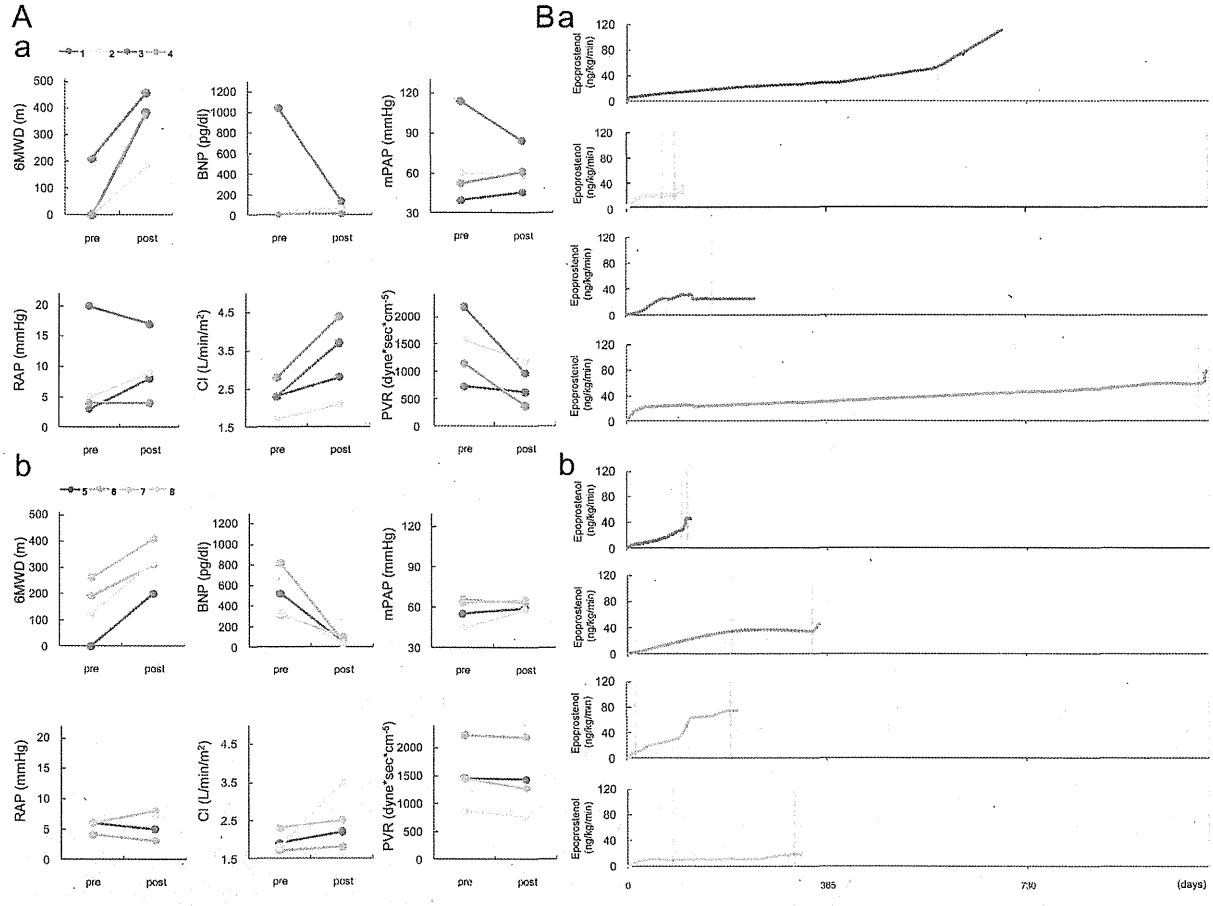


**Figure 2.** Changes in parameters and dose titration of epoprostenol. (A) Parameters before and after epoprostenol therapy were compared in patients without lung transplantation (a) and patients who underwent living-donor lobar lung transplantation (LDLLT) (b). Data after epoprostenol treatment were obtained at the time when patients achieved the best values for the cardiac index (CI) by right heart catheterization. The 6-min walk distance (6MWD), plasma concentrations of brain natriuretic peptide (BNP), and CI were significantly improved by epoprostenol therapy. mPAP, mean pulmonary artery pressure; RAP, right atrial pressure; PVR, pulmonary vascular resistance. (B) Dose titration of epoprostenol of each patient is shown (patients without lung transplantation (a) and patients who underwent LDLLT (b)). Red dotted lines indicate the time when patients achieved the best values for CI. Gray dotted lines indicate the time when patients showed deterioration.

**Table 3. Epoprostenol Therapy and Associated Therapy**

| Patient no. | After epoprostenol therapy | | | | Final | |
|---|---|---|---|---|---|---|
| | Time from initiation (days) | Dose ($ng \cdot kg^{-1} \cdot min^{-1}$) | Bosentan (mg/day) | Sildenafil (mg/day) | Time from initiation (days) | Dose ($ng \cdot kg^{-1} \cdot min^{-1}$) |
| 1 | 82 | 12.5 | – | – | 685 | 110.5 |
| 2 | 66 | 15.0 | – | – | 102 | 33.7 |
| 3 | 70 | 24.9 | – | 60 | 234 | 32.0 |
| 4 | 708 | 46.3 | – | – | 1,063 | 79.2 |
| Mean of patients 1–4 | 231.5±158.9 | 24.7±7.7 | | | 528.0±216.6 | 63.9±19.0 |
| 5 | 98 | 45.0 | – | – | 115 | 46.0 |
| 6 | 193 | 34.9 | – | – | 351 | 45.4 |
| 7 | 14 | 7.5 | 125 | 40 | 202 | 75.2 |
| 8 | 82 | 9.0 | 250 | – | 318 | 21.0 |
| Mean of patients 5–8 | 96.8±36.9 | 24.1±9.4 | | | 246.5±54.2 | 46.7±11.1 |

After epoprostenol therapy, at the time when patients achieved the best values for cardiac index; final, at the time of lung transplantation or death; time from initiation, time from initiation of epoprostenol therapy; dose, dose of epoprostenol.

centrilobular congestion were observed at low magnification, and characteristic dilatation of alveolar capillaries was observed at a higher magnification (Figure 1A). Hemosiderin-laden macrophages were often observed in the alveolar space. PVOD was characterized by marked stenosis and occlusion of small intrapulmonary veins (Figure 1B). Vessel walls were thickened by intimal fibrous proliferation. In patients 4 and 5, invasive proliferation of capillaries were also observed in the walls of bronchi and arteries, leading to the diagnosis of PCH (Figure 1C). These capillaries were engorged and tortuous.

Four patients successfully underwent LDLLT and the remaining 4 patients had no suitable living donors of the lung and finally died while awaiting cadaveric lung transplantation. The causes of death were respiratory failure or concomitant respiratory infection. No patient died from adverse effects of epoprostenol itself.

### Patient Characteristics Before Epoprostenol Therapy

Patient characteristics before epoprostenol therapy are shown in Table 2. All patients were in WHO functional class III and IV. The 4 patients who were in WHO functional class IV could not walk because of severe oxygen desaturation at baseline. The other 4 patients in WHO functional class III could only walk approximately 200 m (Figure 2A). Plasma BNP levels were not always elevated. Three patients showed low BNP levels in spite of the severity of their general condition and inability to walk. For the pulmonary function test, 2 patients showed mild restrictive defects (62% and 72%), and another patient showed a mild obstructive defect (65%). Overall, lung function was within normal limits (%vital capacity: 86.4±6.3%; forced expiratory volume at 1 s: 77.4±3.1%) except for low %DLco (45.8±8.6%). All patients manifested pulmonary hypertension with a mean PAP of 61.5±8.1 mmHg on right heart catheterization. Pulmonary capillary wedge pressure and right atrial pressure were within the normal range in all patients. In 4 patients, the cardiac index was lower than 2.0 $L \cdot min^{-1} \cdot m^{-2}$.

**Table 4. Radiographic Findings at Baseline and After Epoprostenol Therapy**

| Radiographic findings | PVOD and PCH (n=8) |
|---|---|
| **Baseline** | |
| Dilated pulmonary arteries | 8 |
| Kerley B lines | 2 |
| Interstitial infiltrates | 8 |
| Ground-glass opacities | 7 |
| Pleural effusion | 2 |
| Interlobular thickening | 8 |
| Lymphadenopathy | 3 |
| **After epoprostenol therapy** | |
| Increase in pleural effusion | 3 |
| Thickened interlobular septae | 8 |
| Deterioration of ground-glass opacities | 8 |

Data indicates the number of patients.
PVOD, pulmonary veno-occlusive disease; PCH, pulmonary capillary hemangiomatosis.

### Efficacy of Epoprostenol Therapy

Patients were cautiously treated with epoprostenol for 387.3±116.3 days (range, 102–1,063 days) (Table 3; Figure 2B). The maximum dose of epoprostenol given was 55.3±10.7 $ng \cdot kg^{-1} \cdot min^{-1}$ (range, 21.0–110.5 $ng \cdot kg^{-1} \cdot min^{-1}$). Patients who had no chance to undergo a lung transplantation had epoprostenol therapy applied for 528.0±216.6 days and the maximum dose was 63.9±19.0 $ng \cdot kg^{-1} \cdot min^{-1}$. The best value for cardiac

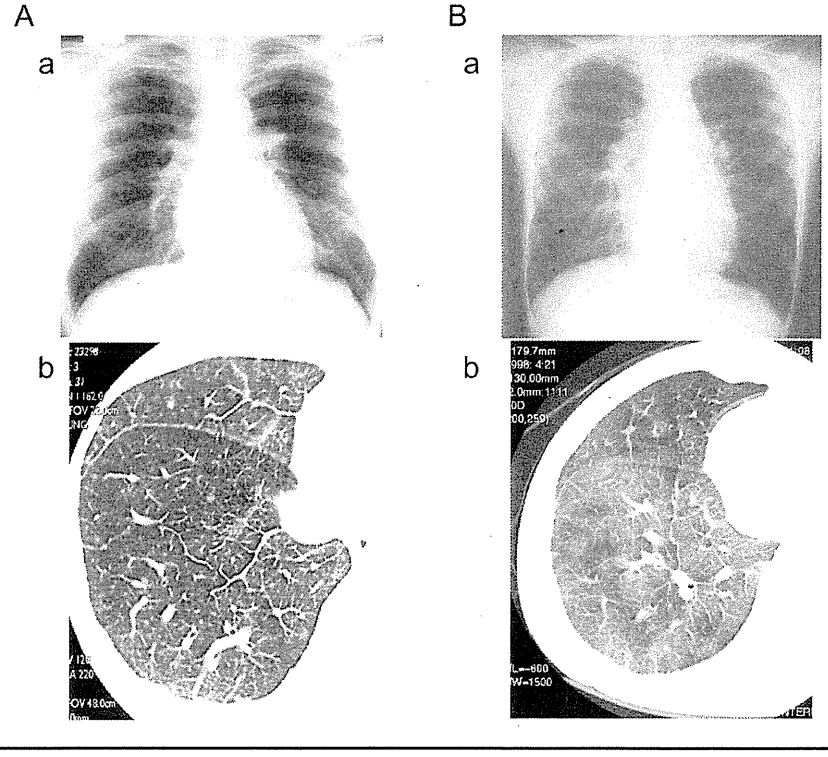


**Figure 3.** Representative radiographic findings. (A) Radiographic findings of pulmonary veno-occlusive disease (patient 1). (a) A chest radiograph shows a prominent main pulmonary artery and interstitial infiltrates. (b) A chest computed tomography (CT) scan of the right lobe shows multiple thickened interlobular septal lines, ground-glass opacities, and pleural effusion. (B) Radiographic findings of pulmonary capillary hemangiomatosis (patient 4). (a) A chest radiograph shows a prominent main pulmonary artery and main truncus of the right pulmonary artery. Faint nodular opacities and numerous Kerley B lines are also shown. (b) A chest CT scan shows diffuse, ill-defined, ground-glass opacities, and interlobular septal thickening is less prominent.

Table 5. Flow of Supplemental Oxygen Required Before and After Starting Epoprostenol Therapy

| Patient no. | Baseline | Best | Later |
|---|---|---|---|
| 1 | 3 | 2 | 9 |
| 2 | 2 | 5 | 8 |
| 3 | 3 | 2 | 15 |
| 4 | 4 | 4 | 10 |
| 5 | 2 | 3 | 12 |
| 6 | NA | 3 | 10 |
| 7 | 3 | 3 | 12 |
| 8 | 3 | 4 | 10 |
| P value | | NS | <0.01 |

Data indicate the flow of supplemental oxygen (L/min).
Repeated-measures analysis of variance with Bonferroni correction was performed. P values indicate "best" and "later" values compared with the "baseline" value.
Baseline, before starting epoprostenol therapy; best, at the time when patients achieved the best values for cardiac index; later, maximum oxygen flow required while the dose of epoprostenol was increased later.

index was obtained at 164.1±79.7 days after initiation of epoprostenol with a dose of 24.4±5.6 ng·kg$^{-1}$·min$^{-1}$.

After the application of epoprostenol, the WHO functional class improved at least temporarily to class II or III in all patients. The mean 6-min walk distance significantly increased from 97.5±39.2 to 329.4±34.6 m (P<0.001) (Table 2; Figure 2A). As mentioned above, plasma levels of BNP were not always elevated at baseline. In patients who had high BNP levels prior to epoprostenol therapy, BNP levels were significantly reduced after therapy. In total, the mean BNP levels were significantly reduced from 381.3±136.8 to 55.2±14.4 pg/ml (P<0.05). The mean cardiac index significantly improved from 2.1±0.1 to 2.9±0.3 L·min$^{-1}$·m$^{-2}$ (P<0.05). However, the mean PAP and right atrial pressure did not change between before and after epoprostenol therapy. Although mixed venous oxygen saturation was increased and pulmonary vascular resistance was decreased after epoprostenol therapy, these differences were not statistically significant.

### Associated Therapy

Associated therapy before and after epoprostenol therapy is shown in Tables 2 and 3. At baseline, anticoagulation and diuretics were used in all patients, digitalis was given in 4 patients (patients 2, 5, 6, and 7), and no calcium channel blockers were used in any of these patients. An endothelin receptor antagonist, bosentan, was used in 2 patients (patient 7: 125 mg/day; patient 8: 250 mg/day) and a phosphodiesterase 5 inhibitor, sildenafil, was used in 2 patients (patient 3: 60 mg/day; patient 7: 40 mg/day). The doses of bosentan and sildenafil were unchanged during epoprostenol therapy. Catecholamines were not used at the time when patients achieved the best values for the cardiac index. Anticoagulation was discontinued in 2 patients (patients 3 and 4) based on our previous report regarding the risk of alveolar hemorrhage induced by concomitant use with epoprostenol.[13] Digitalis was stopped in patient 5 who manifested bradycardia. All other medications were unchanged after epoprostenol therapy.

### Radiographic Changes and Oxygen Supplementation During Epoprostenol Therapy

All 8 patients manifested atypical radiographic features as IPAH at baseline (Table 4; Figure 3). Their chest radiographs revealed not only dilated pulmonary arteries and enlargement of the heart, but also peripheral interstitial infiltrates in both lung fields, and sometimes prominent septal lines. High-resolution CT scans showed pleural effusion, thickened interlobular septa, bilateral ground-glass opacities, and a mosaic pattern of lung attenuation. Lymphadenopathy in the mediastinum, which is sometimes observed as a reactive adenopathy in PVOD, was detected in 1 patient with PVOD and 2 patients with PCH. After initiation of epoprostenol therapy, all patients' chest X-rays or CTs showed thickened interlobular and intralobular septae and an increased density of interstitial opacities. Three of them also showed an increase in pleural effusion. At that time, we temporarily stopped increasing the dose of epoprostenol and added diuretics and/or intravenous infusion of catecholamines. After congestion improved, we started to titrate the dosage of epoprostenol again.

Before epoprostenol therapy, patients required oxygen supplementation with 2.9±0.3 L/min (Table 5). At the time when patients achieved the best values for cardiac index, patients needed 3.3±0.4 L/min of supplemental oxygen. As the dose of epoprostenol was increased, patients showed deterioration of oxygen desaturation and an increase in interstitial infiltrates on chest X-rays. They finally needed an oxygen supplement at a significantly higher flow (10.8±0.8 L/min) than they did before epoprostenol therapy (P<0.01).

## Discussion

Among a variety of diseases that can lead to pulmonary hypertension, PVOD and PCH are especially rare, and their classification has been changed at all the World Symposiums on Pulmonary Hypertension. In the previous classification of pulmonary hypertension, they were categorized in a subgroup of pulmonary arterial hypertension, termed "pulmonary arterial hypertension associated with significant venous or capillary involvement".[2] In the most recent Dana Point classification, these diseases are classified as Group 1', similar to but with some differences from Group 1, because of their similarities in histological changes, clinical presentations, risk factors and having shared mutations in the BMPR2 gene, similar to that for IPAH.[3]

The prognosis of PVOD and PCH is still unknown because of the rareness of the disease. It is believed to be poor, with most patients with PVOD dying within 2 years from the initial presentation.[7] Most PCH patients rapidly progress to death over several months of the clinical disease.[14] In the last decade, PAH-targeted drugs have improved the survival of patients with PAH.[6,15,16] However, no medical treatment has been proven to improve the survival of patients with PVOD and PCH. Therefore, patients with PVOD and PCH have a higher mortality and a lower chance of survival compared with patients with IPAH.

Currently, lung transplantation is the only method to cure these diseases and patients who desire it are placed on the list for lung transplantation as soon as possible.[4] However, there are few organ donors available to undergo cadaveric lung transplantation. In Japan, where organ transplantation has been recently introduced, chances of transplantation are very limited and the mean waiting time for lung transplantation is reported to be approximately 3 years. In most cases, it is difficult for patients to survive for this long period of time considering their poor prognosis. Although LDLLT is expected to be an alternative for cadaveric lung transplantation, there are more strict criteria for donors of LDLLT.[17,18] Not all patients and their families who desire to receive lung transplantation can

undergo LDLLT. A therapeutic option is required for patients waiting for a suitable donor or for those who are not candidates for lung transplantation.

Continuous intravenous infusion of epoprostenol has been reported to improve the prognosis of IPAH.[6,19] However, its indication for PVOD and PCH is still controversial. Some reports have cautioned against the possibility of causing massive pulmonary edema by application of epoprostenol for patients with PVOD or PCH.[9,10] Application of epoprostenol for PVOD or PCH might be unsuccessful because when the pulmonary arterioles dilate and resistance of the pulmonary veins remains fixed, transcapillary hydrostatic pressure might increase and pulmonary edema might occur.[20] In contrast, some patients with PVOD have been reported to show temporary amelioration by application of epoprostenol.[7,8] There is 1 case report that showed that long-term epoprostenol therapy improved exercise capacity and pulmonary hemodynamics in PVOD.[8] The authors concluded that in this case, the administration of epoprostenol played a role in the regulation of vascular tone in pulmonary venules rather than in the pulmonary arteries. Detailed hemodynamic measurements showed that microvascular pressures initially increased during an infusion of no more than $6\,ng \cdot kg^{-1} \cdot min^{-1}$ of epoprostenol, but at higher doses, cardiac output increased and the calculated pulmonary vascular resistance decreased.[21] To the best of our knowledge, there are no reports that have described patients with PCH being successfully treated with epoprostenol.

We administered epoprostenol to 8 patients with PVOD or PCH because they had no other therapeutic option besides lung transplantation. In our cases, we cautiously administered epoprostenol, starting with a low dose. When we increased the dose of epoprostenol too quickly, an imbalance of dilatation between pulmonary arterioles and veins occurred. However, if we slowly increased the dose in a step-wise manner and used diuretics or inotropes as necessary, the transcapillary hydrostatic pressure decreased and we could avoid severe pulmonary congestion.

For the successful treatment of PVOD and PCH with epoprostenol, early recognition and diagnosis of PVOD/PCH are essential in addition to the careful application of epoprostenol. A lung biopsy is the only method of definitively diagnosing PVOD and PCH. However, in most cases, it is difficult to perform a lung biopsy because of the severity of the patients' condition. This is why it is important to clinically diagnose PVOD/PCH with available data and results of examinations. It is vital to be aware of poor oxygenation, low DLco, and distinct radiographic findings to diagnose or suspect PVOD and PCH.[20,22] In the present study, all patients presented with marked oxygen desaturation on exertion and a severe decrease in DLco. Their chest radiographs and high-resolution CT scans revealed radiographic findings that were characteristic for PVOD and PCH, but not IPAH (Table 4; Figure 3).[14,23] Early recognition of PVOD/PCH in patients with pulmonary hypertension is possible based on these clinical and radiographic characteristics. This might lead to careful introduction and dose adjustment of epoprostenol and to successful treatment of these complicated diseases.

The present study showed that as a result of epoprostenol therapy, clinical and hemodynamic data were improved (Table 2; Figure 2), at least temporarily. All patients were critically ill before starting epoprostenol therapy. The mean 6-min walk distance, which is reported to correlate well with the prognosis in IPAH, was significantly increased after therapy. Our data showed that epoprostenol significantly improved exercise capacity and increased cardiac output of patients with PVOD or PCH, but did not decrease PAP and right atrial pressure, which are known to determine the survival of IPAH.[24] This might be one of the reasons why patients eventually showed deterioration. Most patients showed maximal improvement within half a year after starting epoprostenol therapy. In some cases, with cautious control of epoprostenol therapy, there is a possibility of longer survival than previously reported. The dose of epoprostenol given at the time when patients showed maximal improvement in clinical status was $24\,ng \cdot kg^{-1} \cdot min^{-1}$, regardless of whether they could undergo LDLLT. Although they could walk further in the 6-min walk test because of increased cardiac output with epoprostenol therapy, patients showed deterioration of interstitial infiltration in chest X-rays and CT scans and needed a higher flow of supplemental oxygen. Considering severe oxygen desaturation and limited prognosis with epoprostenol therapy, further studies are required to determine better therapeutic strategies to treat PVOD and PCH.

## Conclusions

We applied epoprostenol treatment to 8 patients with atypical clinical and radiographic findings such as IPAH. Histological findings revealed that 6 patients had PVOD and the other 2 patients had PCH. Epoprostenol was applied at a higher dose and for a longer period than previously reported cases, and worked as a bridge to lung transplantation for 4 patients. It was also applied in 4 patients who had no chance to undergo lung transplantation. All patients showed temporary amelioration in WHO functional class, exercise capacity, and cardiac index with long-term epoprostenol therapy. When patients are suspected of having PVOD or PCH by characteristic clinical and radiographic findings, careful application of epoprostenol can be considered as a bridge to lung transplantation or as the only method to improve their clinical condition because they have no other therapeutic options.

## References


1. Fishman AP. Clinical classification of pulmonary hypertension. *Clin Chest Med* 2001; **22:** 385–391, vii.
2. Simonneau G, Galie N, Rubin LJ, Langleben D, Seeger W, Domenighetti G, et al. Clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2004; **43:** 5S–12S.
3. Simonneau G, Robbins IM, Beghetti M, Channick RN, Delcroix M, Denton CP, et al. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2009; **54:** S43–S54.
4. McLaughlin VV, Archer SL, Badesch DB, Barst RJ, Farber HW, Lindner JR, et al. ACCF/AHA 2009 Expert Consensus Document on Pulmonary Hypertension: A report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association: Developed in collaboration with the American College of Chest Physicians, American Thoracic Society, Inc., and the Pulmonary Hypertension Association. *Circulation* 2009; **119:** 2250–2294.
5. Higenbottam T, Butt AY, McMahon A, Westerbeck R, Sharples L. Long-term intravenous prostaglandin (epoprostenol or iloprost) for treatment of severe pulmonary hypertension. *Heart* 1998; **80:** 151–155.
6. Barst RJ, Rubin LJ, Long WA, McGoon MD, Rich S, Badesch DB, et al. A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension: The primary pulmonary hypertension study group. *N Engl J Med* 1996; **334:** 296–302.
7. Holcomb BW Jr, Loyd JE, Ely EW, Johnson J, Robbins IM. Pulmonary veno-occlusive disease: A case series and new observations. *Chest* 2000; **118:** 1671–1679.
8. Okumura H, Nagaya N, Kyotani S, Sakamaki F, Nakanishi N, Fukuhara S, et al. Effects of continuous IV prostacyclin in a patient with pulmonary veno-occlusive disease. *Chest* 2002; **122:** 1096–1098.
9. Palmer SM, Robinson LJ, Wang A, Gossage JR, Bashore T, Tapson VF. Massive pulmonary edema and death after prostacyclin infusion

in a patient with pulmonary veno-occlusive disease. *Chest* 1998; **113:** 237–240.
10. Humbert M, Maitre S, Capron F, Rain B, Musset D, Simonneau G. Pulmonary edema complicating continuous intravenous prostacyclin in pulmonary capillary hemangiomatosis. *Am J Respir Crit Care Med* 1998; **157:** 1681–1685.
11. Montani D, Jais X, Price LC, Achouh L, Degano B, Mercier O, et al. Cautious epoprostenol therapy is a safe bridge to lung transplantation in pulmonary veno-occlusive disease. *Eur Respir J* 2009; **34:** 1348–1356.
12. Barst RJ, McGoon M, Torbicki A, Sitbon O, Krowka MJ, Olschewski H, et al. Diagnosis and differential assessment of pulmonary arterial hypertension. *J Am Coll Cardiol* 2004; **43:** 40S–47S.
13. Ogawa A, Matsubara H, Fujio H, Miyaji K, Nakamura K, Morita H, et al. Risk of alveolar hemorrhage in patients with primary pulmonary hypertension: Anticoagulation and epoprostenol therapy. *Circ J* 2005; **69:** 216–220.
14. Almagro P, Julia J, Sanjaume M, Gonzalez G, Casalots J, Heredia JL, et al. Pulmonary capillary hemangiomatosis associated with primary pulmonary hypertension: Report of 2 new cases and review of 35 cases from the literature. *Medicine (Baltimore)* 2002; **81:** 417–424.
15. Fukumoto Y, Shimokawa H. Recent progress in the management of pulmonary hypertension. *Circ J* 2011; **75:** 1801–1810.
16. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, et al. Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. *Circulation* 2010; **122:** 156–163.
17. Starnes VA, Bowdish ME, Woo MS, Barbers RG, Schenkel FA, Horn MV, et al. A decade of living lobar lung transplantation: Recipient outcomes. *J Thorac Cardiovasc Surg* 2004; **127:** 114–122.
18. Date H, Kusano KF, Matsubara H, Ogawa A, Fujio H, Miyaji K, et al. Living-donor lobar lung transplantation for pulmonary arterial hypertension after failure of epoprostenol therapy. *J Am Coll Cardiol* 2007; **50:** 523–527.
19. Akagi S, Nakamura K, Miyaji K, Ogawa A, Kusano KF, Ito H, et al. Marked hemodynamic improvements by high-dose epoprostenol therapy in patients with idiopathic pulmonary arterial hypertension. *Circ J* 2010; **74:** 2200–2205.
20. Mandel J, Mark EJ, Hales CA. Pulmonary veno-occlusive disease. *Am J Respir Crit Care Med* 2000; **162:** 1964–1973.
21. Davis LL, deBoisblanc BP, Glynn CE, Ramirez C, Summer WR. Effect of prostacyclin on microvascular pressures in a patient with pulmonary veno-occlusive disease. *Chest* 1995; **108:** 1754–1756.
22. Frazier AA, Franks TJ, Mohammed TL, Ozbudak IH, Galvin JR. From the archives of the AFIP: Pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis. *Radiographics* 2007; **27:** 867–882.
23. Resten A, Maitre S, Humbert M, Rabiller A, Sitbon O, Capron F, et al. Pulmonary hypertension: CT of the chest in pulmonary venoocclusive disease. *AJR Am J Roentgenol* 2004; **183:** 65–70.
24. D'Alonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, et al. Survival in patients with primary pulmonary hypertension: Results from a national prospective registry. *Ann Intern Med* 1991; **115:** 343–349.