

## **Hypoxic Pulmonary Vascular Reactivity with Advancing Age in Rats**

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Despite many investigations, the mechanism of hypoxic pulmonary vasoconstriction (HPVC) has not been clearly elucidated. The hypoxic-pressor response occurs in isolated perfused lung, the basic mechanism of hypoxic vasoconstriction does not require systemic neural or humoral input (1). However, two types of hypotheses have been proposed to explain the main mechanism of HPVC. First, hypoxia may elicit pulmonary vasoconstriction via a direct action on the pulmonary vascular smooth muscle (2). Second, some unknown cell type senses hypoxia and produces and/or releases a constrictive mediator, which then stimulates the smooth muscle to contract (2). There is little information on changes in pulmonary vascular reactivity-induced by aging (3,4). We now describe an increased pulmonary vascular responses to alveolar hypoxia and Angiotensin II (AII) administration in old rats compared with young rats. The comparison studies were performed with isolated perfused lung with blood. Also, this study was made the measurements of pulmonary and systemic arterial blood pressures at

rest in conscious these rats.

\* \* \* \* \*

The lungs were obtained from male Sprague-Dawley (SD) rats with a body weight  $520 \pm 50$  g, obtained as young aged (2-months of age) and old aged (12-months of age). The isolated perfused lung with blood (Fig. 1) was allowed to equilibrate for 20 min under normoxic condition (21.0% O<sub>2</sub>, 5.1% CO<sub>2</sub>, N<sub>2</sub> Balance) prior to beginning experimentaion. The protocol was identical for each rat lung preparation and was

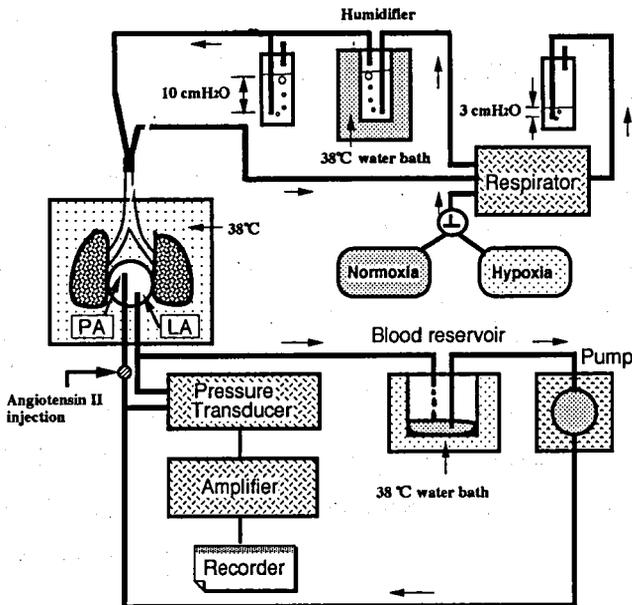


Fig.1 Measurement methods of pulmonary hemodynamics in isolated-perfused rats lungs.

PA: Pulmonary artery, LA: Left atrium, Normoxia: 21.0% O<sub>2</sub>, 5.1% CO<sub>2</sub>, N<sub>2</sub> Balance, Hypoxia: 3.0% O<sub>2</sub>, 5.1% CO<sub>2</sub>, N<sub>2</sub> Balance

patterned after previous studies (5). A 5 min hypoxix (3% O<sub>2</sub>, 5.1% CO<sub>2</sub>, N<sub>2</sub> Balance) exposure and 5 min normoxic recovery period was followed by an intra arterial injection of Angiotensin II (A II, Sigma) at a dose of 0.2  $\mu$ g. The Ppa returned to the baseline level 10 min after A II injection.

On the other hand, male SD rats (old and young aged-rats) weighing 420 to 620 g were anesthetized with ketamine hydrochloride (70 mg/ kg, ip). Hand-made catheters were placed in the carotid artery, jugular veins, and pulmonary artery as previously described (Fig.2,3)(6). Six hours after the catheter placement, rats were studied that mean systemic and pulmonary artery pressures were measured by pressor transducers (Nihon Kohden, TAP-300). After measurements of baseline hemodynamics, all animals were then killed by overdose pentobarbital sodium (50mg/ kg, ip). The ventricle was removed en bloc. following the method of Fulton et al..(7), right ventricular free wall (RV) and left ventricle together with septum (LV+S) were weighed separately.

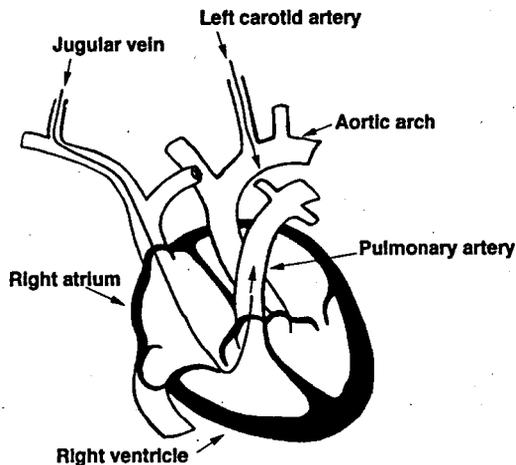


Fig.2 Measurements of pulmonary and systemic arterial blood pressures.

We express their weights as the ratio  $RV/(LV+S)$ .

\* \* \* \* \*

Fig.4 shows the Ppa measured for the old and young groups on

### Measurement of pulmonary arterial pressure

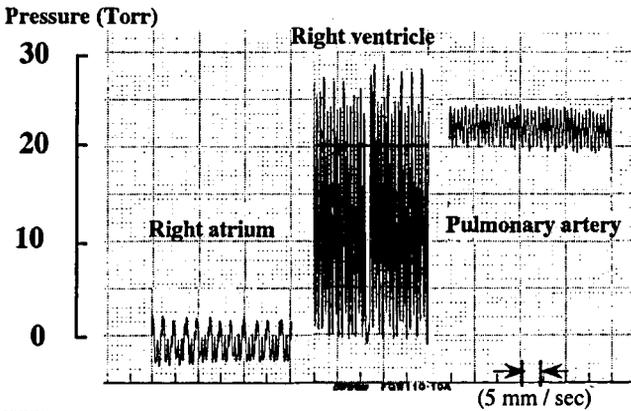


Fig.3 The waves of each blood pressure.

Pulmonary arterial pressure (Torr)

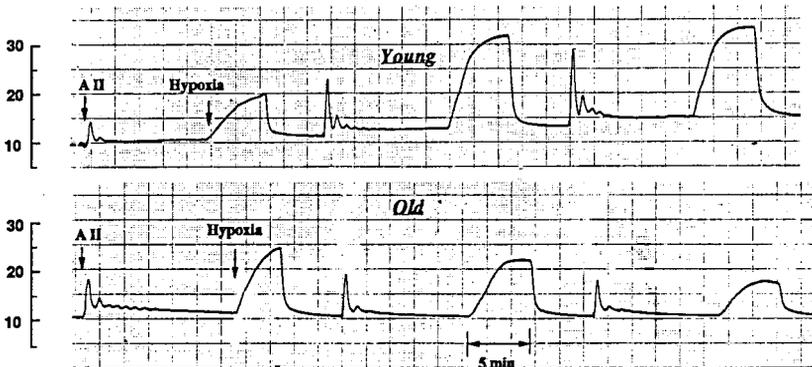
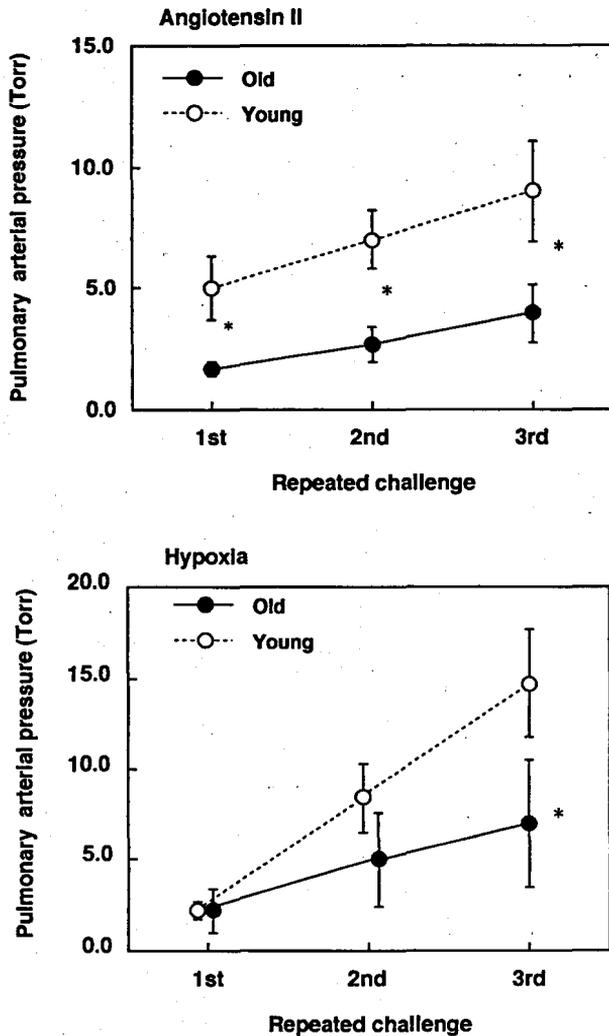


Fig.4 Representative tracings of alternating pulmonary arterial pressure (Ppa) induced by hypoxic and Angiotensin II challenges.



**Fig.5** Pulmonary vascular pressor responses to repeated challenges with alveolar-hypoxia and bolus injection of Angiotensin II (0.2  $\mu\text{g}/0.1\text{ml}$  saline).  
 \*Statistically significant differences from old rats values ( $p < 0.05$ )

isolated perfused lung with blood. This figure shows an example of three pairs of hypoxic vasoconstriction each time followed by A II and hypoxia-induced vasoconstrictions. Isolated perfused lung from old-age rats had reduced vasoconstriction in comparison with lung from young rats, when exposed alveolar hypoxia. Fig. 5 compares A II-induced pulmonary vasoconstriction in the old and the young groups. Aging enhanced pulmonary vasoreactivity induced by A II. Fig. 6 shows aging factor

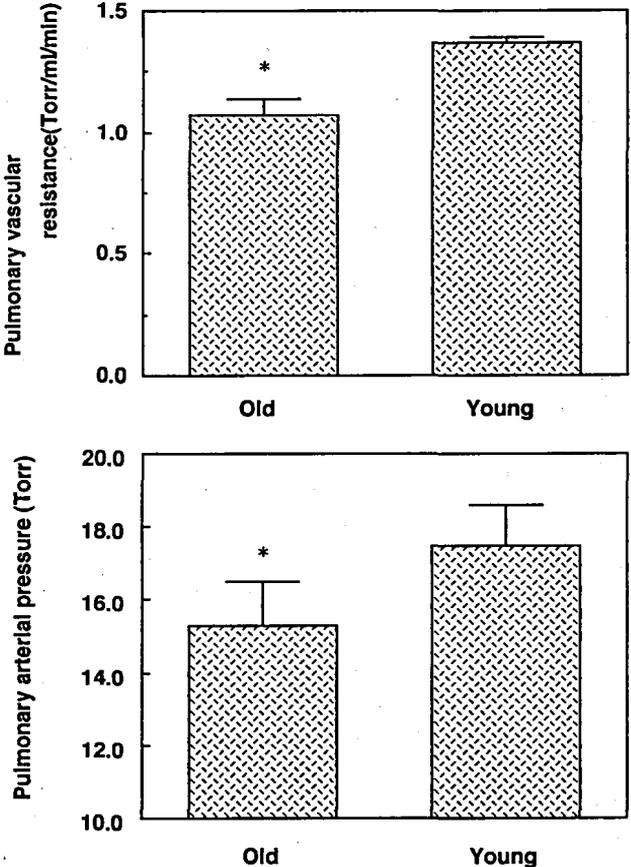


Fig.6 Pulmonary vascular pressures and resistances with alveolar-normoxia in old and young rats. \*p<0.05

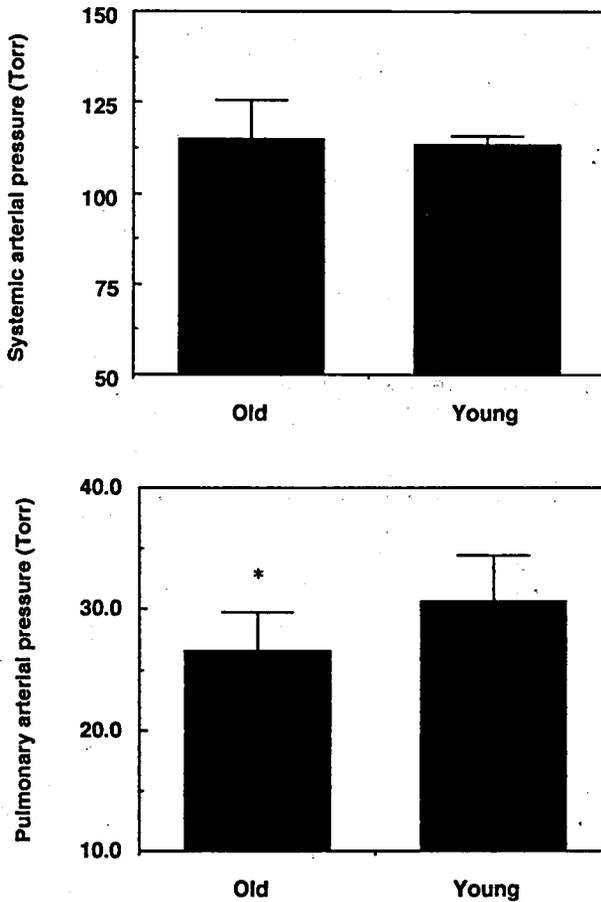


Fig.7 Pulmonary and systemic arterial pressures at rest in conscious old and young rats.  
\* $p < 0.05$

decreased the pulmonary vascular resistance during exposure to normoxia. Figs. 7 and 8 appears, in catheter-implanted rats, aging was decreased the mean pulmonary arterial pressure and pulmonary arterial pressure to systemic arterial pressure ratio ( $P_{pa}/P_{sa}$ ), while not affecting systemic artery pressure. There were no significant

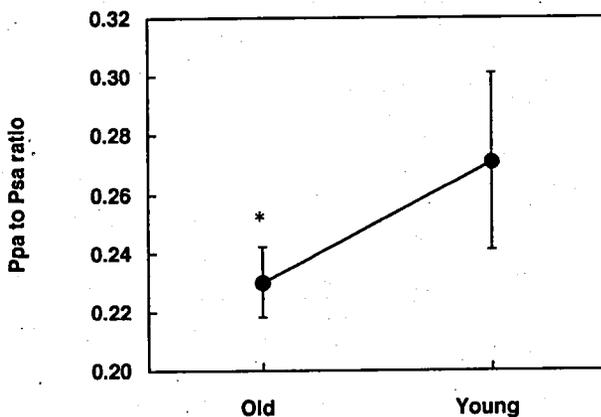


Fig.8 Pulmonary arterial pressure to systemic arterial pressure ratio in conscious old and young rats.  
\* $p < 0.05$

Table 1 Body weights and ventricular weights in old and young rats.

	Old	Young
Body weight(g)	540±8.5	425.5±3.4*
Right ventricle (mg/100g BW)	55.2±2.8	57.7±3.5
Left ventricle with septum (mg/100g BW)	260.0±25.8	261.3±9.4
RV/(LV+S)	0.274±0.030	0.286±0.022

\* $p < 0.05$  vs. Old rats

changes in the right ventricular weight (RV), left ventricular weight with septum (LV+S) and RV to LV+S ratio (RV/(LV+S)) in old and young rats (Table 1).

\* \* \* \* \*

In previous investigation, the infant rats will always have fewer arteries than the adults (8). The infant are left with a similar decrease in arterial concentration and increase in medial wall thickness as the adults, they have more peripheral extension of muscle. Older rats had smaller right ventricular hypertrophy than younger, but the younger rats were studied older than our group and perhaps represent an intermediate group in which the response is blunted. There was a tendency for greater impairment of hypoxic pulmonary vasoconstriction in the aging rats. The aging rats exhibited a different morphological response to altitude exposure. Smooth muscle hypertrophy differences between young and aging rats is not apparent. It is apparent that the aging rats exhibited a markedly different pulmonary vascular morphologic response to chronic hypoxia (8).

In contrast to the numerous studies of systemic vessels, the effects of aging on pulmonary vascular responsiveness-induced by hypoxia has been somewhat limited. Fleish and Hooker reported a reduction in the dilation produced by isoproterenol with advancing age in rabbit and rat pulmonary arteries (9). Also,  $\beta$ -adrenoreceptor activity in the rabbit pulmonary artery has been shown to increase with age (10). Blood vessel undergo a number of alterations during the aging process, including in structure (11), enzyme activities (12), and responsiveness to drug (9). A decrease in  $\beta$ -adrenoreceptor activity in arterial smooth muscle with increasing age has been repeatedly demonstrated (12). Also, aortas from 12-month-old rats contracted less to norepinephrine, serotonin, and KCl than aortas from 2-month-old rats. Lung isolated

from 12 month-old-rats exhibited reduced vascular response to the vasoconstrictor agents Angiotensin II and hypoxia. The reduced hypoxia-induced pulmonary vasoconstriction in the present aging rats may have been due to a membrane or metabolic alteration, although the enhanced synthesis and release of a pulmonary vasodilator cannot be discounted (13). The pulmonary vascular hyporeactivity with advancing age is suggested.

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## ラットの加齢にともなう低酸素性肺血管収縮反応

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要旨：本研究は、加齢にともなう低酸素性肺血管収縮の変化について検討することで、中高年層における高地肺水腫発症の危険性を探ることとした。これらを検討するために、ラットも用い摘出灌流肺装置により低酸素およびA IIによる肺血管収縮反応を直接的に観察することとした。また、覚醒安静時の肺動脈圧(Ppa)および体血圧(Psa)も測定した。Sprague-Dawleyラットを用い、一方は若年ラット(Y群)の2ヶ月令10匹、他方は加齢ラット(O群)の12ヶ月令10匹とした。Fig.1に示すような摘出灌流肺装置を用い、肺循環動態を観察するために、肺換気は21.0% O<sub>2</sub>, 5.1% CO<sub>2</sub>, N<sub>2</sub> Balanceにし、Angiotensin II (0.2 μg/0.1ml)を投与し、10分間の肺血管反応および肺に低酸素ガス(3.1% O<sub>2</sub>, 5.1% CO<sub>2</sub>, N<sub>2</sub> Balance)を5分間換気し肺血管反応をみた。カテーテル挿入による肺動脈圧の測定実験をおこなうため、O群5匹とY群5匹を用いた。PpaおよびPsa測定は、安静時標高760m(辰野町)で行った。Hypoxia換気時およびA II投与のPpaは、O群がY群より有意(p<0.05)に低値を示した。また、PpaおよびPVRは、O群がY群より有意(P<0.05)に低値を示した。覚醒時のPpaは、O群がY群より有意(p<0.05)に低値を示した。しかし、Psaは、O群とY群の間には有意差がなかった。右心室重量(RV)、左心室重量(LV+S)およびRV/(LV+S)には、O群とY群には差がなかった。しかし、Y群は、O群に比較して右心室肥大の傾向にあった。今回の実験から、低酸素とAngiotensin IIに対する肺血管収縮反応は、加齢にともない減少することがわかった。このことは、肺血管膜に対する代謝的变化によるかも知れないが、肺血管拡張物質の放出促進とは考えにくく、今後の研究課題としたい。