1. Role of the pulmonary vascular system in cardiovascular and respiratory regulation during microgravity exposure


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4. Summary of Research

Objective and Significance: The objective of this study is to investigate the role of the pulmonary circulation in acute and adaptive responses of cardiovascular and respiratory systems during spaceflight and on return to Earth. To this end, a unique X-ray TV system was modified so as to build a monitor system that enables the direct observation of microvessel (50-80 µm in internal diameter) responses in thick layers of organs in vivo. Subsequently, a micro-angiography method was applied to anaesthetised rats, and the improvement of the selective cannulation technique and the utilisation of a low-viscosity non-ionic hypotonic contrast medium. Applying these methods to anaesthetised rats, results were obtained.

Methods

1) Our unique X-ray TV system was modified to increase its resolution up to 8 µm. The improvement was achieved by replacing the X-ray source by a microfocal (8 µm) X-ray tube and the sensor to a 1-inch II and modifying the sample stage so that it is able to move freely on the X, Y and Z axes. Furthermore, micro-angiography in rats and mice became possible through producing micro-catheters, the improvement of the selective cannulation technique and the utilisation of a low-viscosity non-ionic hypotonic contrast medium. Applying these methods to anaesthetised rats, internal diameter changes of arterioles (50-80 µm in internal diameter) and small arteries (> 100 µm) in the lung and skeletal muscle were measured in vivo. Muscle were measured in vivo.

2) A system was set up for awake mice so that circulatory, ventilatory, metabolic and neural functions of small laboratory animals in real time were developed. After confirming practical performance of the systems by animal experiments, abnormalities of vasomotor functions of pulmonary and skeletal muscle small arteries in the lower extremity due to microgravity exposure were studied using the hindlimb unweighting (HU) rat which is a model system to study cardiovascular dynamics under zero-gravity environment on Earth.

Results

1) A selective angiography technique, in combination with our X-ray TV system, revealed the mechanism of vasomotor regulation through nitric oxide (NO) in rat pulmonary circulation under normoxic and hypoxic conditions. We found that, in normoxic rats, NO derived from eNOS is chiefly involved in the tone regulation of conduit pulmonary arteries, whereas in hypoxic rats, it regulates the tone not only in conduit arteries, but also in the resistance arteries which are the targets of hypoxic vasoconstriction. Moreover, NO derived from iNOS also contributed to inhibiting hypoxic tone elevation in resistance arteries.

2) New findings on the mechanisms of vasomotor regulation in normal pulmonary and skeletal muscle circulation were obtained. The internal diameter of small arteries and arterioles in the skeletal muscles of the lower extremity (gastrocnemial and soleus muscles) and in the lung could be clearly visualised at a geometrical magnification of 4 (total magnification of 48) using angiography of anaesthetised rats. We found that 1) both skeletal muscle small arteries and arterioles have a good vasodilator responsiveness, but the responsiveness is extremely poor in the pulmonary arterioles; 2) in the skeletal muscles, the tone of the small arteries (> 100 µm) is chiefly regulated by the NO dependent vasodilator mechanism, whereas the arteriolar tone is primarily regulated by NO independent vasodilator mechanism (EDHF).

3) Effects of microgravity on the neural and humoral regulation of pulmonary and skeletal muscle microcirculation were elucidated using 3-wk hindlimb unweighting (HU) rats. In the skeletal muscle arteries of the lower extremity, we found that responses to acetylcholine, nitroprusside, sympathetic nerve stimulation and humoral norepinephrine are significantly decreased by HU, except that responses to isoproterenol are unaffected. Vasconstrictor responses to L-NAME were also decreased. The results suggested that the decreased constriction to neural and humoral norepinephrine is not associated with the increase in NO or β-receptor mediated vasodilator function, but is due to a decrease in β-adrenergic constrictor mechanism. Moreover, the data suggested for the first time that HU attenuates arteriolar dilator function due to EDHF. In addition, the decreased responses to nitroprusside suggested a hypofunction of the cyclic GMP pathway. However, it is necessary to consider the possibility that the vascular smooth muscle atrophy in skeletal muscle arteries partly contributes to the vasomotor hypofunction.

In the pulmonary arteries, HU enhanced vasodilator responses due to NO, in contrast to the case of the skeletal muscle arteries. On the other hand, HU attenuated neural and humoral β-adrenergic
and hypoxic constrictor mechanisms. The vasodilator functions mediated by cGMP pathway and $\beta$-receptors were not changed. The attenuation of the $\beta$-adrenergic constrictor mechanism is thought to be caused by a decreased $\beta$-receptor function, a sympathetic nerve hypofunction and/or a secondary effect of an increased NOS function, although this has yet to be determined.

4) Novel findings were obtained on hypoxic response mechanisms in mice.

The newly developed system was applied to control and prostacycline (PGI2) defective mice. We found that PGI2 deficiency alone does not greatly affect acute hypoxic responses of circulation, ventilation and metabolism. However, when combined with an absence of NO, a lack of acute increases in heart rate and ventilation during hypoxia occurred. This led to extremely hazardous conditions in life maintenance.

5) The measurement of pulmonary sympathetic nerve activity in anaesthetised rats was successfully carried out.

Lung specific discharge patterns which differ from those of the kidney were observed. The discharge patterns disappeared when hexamethonium bromide was administered, indicating that these are attributable to postganglionic fibers.

Future achievement towards experiments in space laboratory

Although it is risky to directly relate the data from the HU rats with the deconditioning caused by space environment, our results suggest that orthostatic intolerance on return to Earth could be associated with: 1) vasoconstrictor and vasodilator hypofunctions in small arteries and arterioles of the skeletal muscles of the lower extremity; and 2) reduced blood supplemetning function to the systemic circulation from the pulmonary vascular system which acts as a blood reservoir. It is necessary to extend the experiment to determine if the same results will be obtained by using rats actually reared in an in-space environment. In addition, since the X-ray TV system developed in this study can be applied to the brain, kidneys, liver and intestines, the study can be extended to investigate changes in vasomotor functions of these organs after spaceflight. Furthermore, if the device can be made smaller and lighter, it will be very useful for observing the heart, lungs, digestive tract, and bones in space.

The co-ordination of circulation, respiration and metabolism is essential to life maintenance. However, conventionally, researchers have studied these systems separately. The simultaneous monitoring system of circulation, ventilation and metabolism for small laboratory animals developed in this study enables the analysis of the co-operate action of these functions. This device can be used to evaluate the knowledge gained at the gene and protein level at the whole body level when the transgenic or knockout mouse technique is applied. Because of its compact size, the utilisation of this device in space is realistic. In the future, such a technique might be very useful for the molecular level analyses of mechanisms of acute responses and adaptation of circulation, ventilation and metabolism during spaceflight and on return to Earth.

The technique for measuring the pulmonary sympathetic nerve activity can be further developed for elucidating the importance of the neural regulation of the circulation through the lung. In the future, a practical application of chronic telemetry recording technique for nerve activities in small laboratory animals would be particularly useful.

References


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