The Difference in the Tolerance of Left Ventricular Muscles of Young and Middle-aged Rats to Relative Hypoxia

Sang Chul Lee, M.D. and Byung Moon Han, M.D.

Department of Anesthesiology, Seoul National University College of Medicine, Seoul, Korea

=국문초록=

어린 쥐와 중년쥐의 좌심근의 상대적 저산소증에 대한 내성의 비교연구

서울대학교 의과대학 마취과학교실

이 상 철 · 함 병 문

저산소증에 노출된 경우 연령에 따른 내성의 차이를 살펴보기 위하여 세가지 산소농도(50, 15 and 12.5%)에 무순으로 쥐의 좌심실에서 분리한 유두근을 임의로 노출시키어 이 근육의 등장성(等長性) 수축에 미치는 영향을 비교 관찰하였다. 중년의 쥐들이 오히려 어린 쥐들에 비하여 특히 T_{pd} , $+dp/dt_{max}$ and $RT_{90\%}$ 에서 보진테 더 강한 내성을 보여주었다. 상대적 저산소증으로 인한 수축력 억제 정도의 저산소증의 증가에 의하여 T_{pd} , $+dp/dt_{max}$ 와 $-dp/dt_{max}$ 에서 각기, 어린 쥐들은 -25%에서 -49%로, -22%에서 -45%로, 그리고 -27%에서 -45%로 증가하였으며 중년쥐들의 경우 -14%에서 -38%로, -1%에서 -27%로 또한 -3%에서 -32%로 증가하였다. 또한 $RT_{90\%}$ 에서 보면 산소농도의 감소에 따라 어린 쥐에서 그 증가가 심하였다.

물론 더 많은 연구가 필요하나 이상의 결과로 보아 중년의 쥐들이 어린 쥐들에 비하여 상대적 저산소증에 대하여 더 높은 내성을 나타냈다고 할 수 있으며 실제 임상에서 저산소증에 노출된 어린 환자의 경우 중년의 환자보다 더 심한 심근기능장애를 일으킬 가능성이 있으므로 더욱 빠르고 적절한 조치가 필요할 가능성이 있다고 사료되는 바이다.

Key Words: Tolerance, Relative Hypoxia, Ventricular Muscles

INTRODUCTION

Reduction of extracellular oxygen tension brings a marked effect on myocardial contractility in mammals¹⁾. It has been known that the young of many species appears able to survive in the absence of oxygen for prolonged time period when compared with adults, because heart of the young has more glycogen stores

and a shift in the lactate dehydrogenase isoenzyme pattern. In fetus and newborn, fetal hemoglobin might play a role, too. A relatively large quantities of glycogen preserves both electrical and mechanical activity during hypoxia by anaerobic breakdown of carbohydrates.

Fazekas et al. reported that there is no difference with adult after 17 days of age in rat²⁾ in isometric contraction, while Heller and Whitehorn³⁾ observed that the cross-sectional

area of the papillary muscle increased significantly with age, but isometric properties were not age dependent. Only the velocity and extent of shortening at low preloads were significantly age dependent and the rate phenomena of the contracting muscle are most affected by the aging process in their study. They suggested that there is a possibility that older myocardium may show less tolerance to hypoxia than young ones even though their ages are older than newborns.

It is our clinical impression that myocardial susceptibility to damage from hypoxia is greater in aged patients than younger ones because there is progressive functional decline in cardiovascular system, as well as other organs, with getting old. But there has been no study compared the resistance to hypoxia in a isolated papillary muscle model between young and old rats. The aim of the present investigation was to study the effects of 3 different concentrations (12.5, 25, and 50%) of oxygen on the isolated papillary muscles of young and middle-aged rats, so as to demonstrate in-vitro presumably the increased susceptibility of senescent cardiac muscle to hypoxia.

MATERIALS AND METHODS

Isolated columns of papillary muscle were prepared from left ventricles of rats. 15 papillary muscles were prepared from 2 months old rats (young group: YG) and 15 papillary muscles were prepared from 12~14 months old rats (middle-aged group: MG). Each preparation was placed in an oxygenated (oxygen 95%, carbon dioxide 5%) 80 ml capacity muscle bath containing modified Krebs-bicarbonate solution. The temperature of the bath solution was held constant at 35°C by a thermoregulator (Precision Scientific Co.), and the pH was stabilized at 7.40.

One end of the muscle was fixed, and the other was connected to a force displacement transducer (Grass FT03C) and its output recorded on one channel, while another output was connected to the differentiator (Optic Electronics Inc., model 9009) and recorded on another channel of the recorder (Gould Brush Recorder 2400). Each muscle was stimulated (Grass SD0 Stimulator) with 2×8 mm plate electrodes, placed about 10 mm apart parallel to the muscle, at a frequency of 0.25 Hz with a square wave pulse of 6.0 ms duration and voltages 10% above threshold. The length-tension curve of each preparation was determined after 30 minutes of isometric contractions.

Changes in muscle length measured with a micrometer transformed could be detected to 0.01 mm. The maximum muscle length-tension (the length at the peak of the length-tension curve) was 5.23 ± 1.43 mm (mean $\pm$ S.D.) in YG and 5.71 ± 1.58 mm in MG. The muscle was held at the peak of the length-tension curve for 30 minutes for stabilization, and then peak developed tension (Tpd), resting tension (Tr), maximum rate of tension development ($+dp/dt_{max}$) and relaxation ($-dp/dt_{max}$), time to peak tension (TPT), and relaxation time (RT_{90%}, time for Tpd to decay 90% of maximum) were recorded at a paper speed of 100 mm per second.

At the maximum muscle length of the length-tension curve of each preparation, both groups were exposed to 3 different concentrations of oxygen (12.5, 25, and 50%) in random order. The effects of different oxygen concentrations were computed as the differences between the observed measurements, and control measurements obtained with the control gas mixture of 95% oxygen and 5% carbon following measurement were made in all experiments. Thus the percent changes from the control values of each parameter in each group were obtained. Data

for the groups and their concentrations were compared with multiple comparison with a Duncun test after analysis of variances. We accepted the value of $p < 0.05$ as statistically significant.

RESULTS

Fig. 1. shows a typical tracing of decreasing oxygen concentration on isometric contraction in 2 months old rats(YG), showing dose-related depression of Tpd. The greatest depression was shown at 12.5% oxygen concentration.

The effects of decreasing concentrations of oxygen on isometric contraction showed gradual depression in both age groups in Tpd, $+dp/dt_{max}$ and $-dp/dt_{max}$ (Table 1, Fig. 2).

When we compared each measured parameter with its own control value, there were statistically significant depressions of Tpd, $+dp/dt_{max}$, and $-dp/dt_{max}$ in every oxygen concentration except $+dp/dt_{max}$ and $-dp/dt_{max}$ for 50% oxygen in MG. The depression ranged from -25 to -49

Fig. 1. Typical tracing of decreasing oxygen concentration on isometric contraction in 2 months old rats(young group), showing dose-related depression of peak developed tension. A: 50, B: 25, C: 12.5% of oxygen

Table 1. Effects of Decreasing Concentration of Oxygen on Isometric Contractions of Isolated Rat Heart Muscles

Oxygen conc(%)		Tpd	$+dp/dt_{max}$	$-dp/dt_{max}$	Tr	TPT	RT _{90%}
50	YG	-24.8 ± 12.7	-21.7 ± 9.2	-27.0 ± 10.8	-4.9 ± 14.5	-5.0 ± 6.3	5.5 ± 10.7
	MG	-13.6 ± 10.1	-1.4 ± 9.0	-2.9 ± 16.5	-4.0 ± 3.9	-3.1 ± 9.2	3.7 ± 5.8
25	YG	$-35.8 \pm 12.0^*$	$-30.4 \pm 8.4^*$	$-40.0 \pm 13.3^*$	3.9 ± 11.6	-4.9 ± 9.0	$15.6 \pm 15.0^*$
	MG	-18.9 ± 12.7	$-3.8 \pm 3.8^*$	$-12.8 \pm 6.7^*$	-15.2 ± 12.5	-3.8 ± 7.3	0.6 ± 5.6
12.5	YG	$-48.3 \pm 12.3^{**}$	$-44.9 \pm 13.5^{**}$	$-45.4 \pm 13.4^{**}$	12.1 ± 20.8	-3.6 ± 8.2	$27.3 \pm 22.8^{**}$
	MG	$-37.9 \pm 8.8^*$	$-26.9 \pm 10.3^{**}$	$-31.9 \pm 16.2^{**}$	-4.0 ± 3.4	-11.0 ± 9.5	$10.7 \pm 18.3^*$

Data are mean $\pm$ S.D. of percent changes from control

YG: young group, MG: middle-aged group

*: $P < 0.05$ compared with the value of 50% oxygen concentration

**: $P < 0.05$ compared with the value of 25% oxygen concentration

Tpd: peak developed tension, $+dp/dt_{max}$: maximum rate of tension

Development and relaxation, Tr: resting tension, TPT: time to peak tension, RT_{90%}: time for tension decay 90% of tension

%/-14 to -38%, -22 to 45% / -1 to -27%, and -27 to -45%/-3 to -32% for YG/MG in Tpd, $+dp/dt_{max}$, and $-dp/dt_{max}$ respectively. The degrees of depressions were greater in YG than MG. For Tr, TPT, and $RT_{90\%}$, there was one significant diminishing of Tr in 25% value in MG and no significant change in TPT, but there were significant changes in 12.5 and 25% values in YG.

The differences for Tpd, $+dp/dt_{max}$, and $-dp/dt_{max}$ among the subgroups(12.5, 25, and 50%) were statistically significant except Tpd between 25 and 50% subgroups in MG. For the changes in Tr, TPT, and $RT_{90\%}$ (Table 1, Fig. 2), both age groups showed no significant changes between the subgroups(12.5, 25, and 50%) in Tr and TPT but all the differences of $RT_{90\%}$ between subgroups were significant in YG. In $RT_{90\%}$ of MG, only the difference between 25 and 12.5% was statistically significant(Table 1, Fig. 3).

The depression was significantly greater in

YG than MG in Tpd, $+dp/dt_{max}$ in every oxygen concentration. All of these tendencies became progressively greater with decreasing oxygen concentration. In Tr, TPT, and $RT_{90\%}$, there was no significant difference except of 25% oxygen in $RT_{90\%}$ between two groups(Fig. 2, Fig. 3).

DISCUSSION

During hypoxia, energy for muscle contraction is thought to be derived primarily from the anaerobic breakdown of carbohydrates. In studies of the isolated rat heart, it has been shown that the presence of glucose preserves both electrical and mechanical activity during hypoxia⁴⁾. This might be one of the important reason why many studies observed the better survival of young species during hypoxia than adult. The heart of the young has more glycogen and a shift in the lactate dehydrogenase isoenzyme pattern. Brooks et al. reported that 2-week old rat is an interesting model because,

Fig. 2. Percent changes from control values in peak developed tension(Tpd), maximum rate of tension development and relaxation($\pm dp/dt_{max}$).

■ young group, □ middle-aged group

* $p < 0.05$ compared with young group

A: 50% B: 25% C: 12.5% of oxygen

Fig. 3. Percent changes from control values in resting tension(Tr), time to peak tension(TPT), and time for decay 90% of maximum(RT 90%).

■ young group, □ middle-aged group

*P<0.05 compared with young group

A: 50, B: 25, C: 12.5% of oxygen

while glycogen concentration is similar to the adult, the lactate dehydrogenase isoenzyme profile remains of the fetal type⁵). They told that the altered performance during hypoxia can be assessed in this model independent of the level of endogenous anaerobic substrate.

The relationship between age and the effect of hypoxia on myocardial function have been studied in the intact animal^{16,7)} and in the tissue bath preparation^{1,8)}. Almost of studies were focused on the difference between the very young aged groups, like fetus or newborn, and the adults. But there has been no study comparing the difference in the tolerance between the young groups(older than infant) and the adults. We used the rats aged 2 months which is close to the toddler's age in human, and the rats aged 12 to 14 months which is close to the 5th or 6th decade in human.

By decreasing the concentrations of oxygen on isometric contraction showed gradual depression in both age groups in Tpd, +dp/dt_{max},

and -dp/dt_{max}. This tendency is almost same with the other results^{1,8,9)}.

Su and Friedman⁹⁾ reported that the atria of the young were more sensitive to inhibition of glycolysis by iodoacetate than the adult but no differences were observed in the ability of young and adult ventricular myocardium to generate tension with either oxygen or glucose deprivation. Bing et al⁹⁾ observed that the beneficial effects of glycolysis could be offset by the toxic effect of glycolytic metabolite accumulation if there is 'no flow' situation.

Even though we did not measure the cross-sectional area like Heller and Whitehorn⁹⁾, we could easily recognize that the papillary muscles of the middle-aged rats were much thicker than those of young ones in this experiment. Viewing from the cross sectional area of the muscle for oxygenation, the rate of oxygen delivery to cells along the central axis, or core, of the isolated papillary muscle is determined by the oxygen partial pressure in the external so-

lution, the rate of tissue oxygen utilization, and the diffusion distance for oxygen¹⁰). If the papillary muscle is cylindrical, the oxygen diffusion distance is equal to the muscle radius. Several approaches have been employed to evaluate the oxygen sufficiency of this cardiac muscle preparation, but the methods and conditions have been diverse, and there has been considerable disparity in the values reported for the maximum distance of oxygen penetration or critical radius.

In our study, the degrees of depression were greater in YG than MG for Tpd, $+dp/dt_{max}$, and $-dp/dt_{max}$. The depression ranged from -25 to -49% / -14 to -38% , -22 to 45% / -1 to -27% , and -27 to -45% / -3 to -32% for YG/MG in Tpd, $+dp/dt_{max}$, and $-dp/dt_{max}$ respectively. In addition, the depression was significantly greater in YG than MG. All of these effect were progressively greater with decreasing oxygen concentration. The differences for Tpd, $+dp/dt_{max}$, and $-dp/dt_{max}$ among the subgroups (12.5, 25, and 50%) were statistically significant except Tpd between 25 and 50% subgroups in MG. We may guess that the myocardium of aged rats have more experiences of exposures to hypoxic or anoxic conditions which make them more resistant to relative hypoxia, especially in the condition like our study.

Bing OHL et al.⁹⁾ and Fisher et al.¹¹⁾ observed the inverse relationship between normalized performance and muscle cross-sectional area, which has led previous investigators to suspect the presence of hypoxia of the core in thicker preparations. This investigational result also suggested the possibility of the greater survival rate of young species than aged. And Frezza and Bing OHL¹²⁾ observed the inverse linear relationship between normalized tension development and muscle cross-sectional area in fully oxygenated rat papillary and columnar carneae

muscle studied while contracting isometrically at the apex of the length-tension curve. But they also observed that the thinner muscle showed more depression to hypoxia, which is similar with our results.

We cannot apply our result to clinical practice for human directly because there is a species difference in the tolerance to hypoxia. Bine et al.⁹⁾ reported that improved mechanical performance during hypoxia on the part of turtle heart muscle as compared with the rat is primarily due to the enhanced capacity for anaerobic glycolysis. And Fazekas et al.²⁾ reported that, in the rat at 24 days of age, the oxygen consumption of the brain is like that of the adult while the higher oxygen uptake of the mature dog is developed in the infant when it attains the age of 35 to 50 days.

For the changes in Tr, TPT, and $RT_{90\%}$, there were no drastic changes except of progressively increasing $RT_{90\%}$ with decreasing oxygen concentration which was more significant in YG. In other words, the papillary muscles of young rats recover slowly after exposure to the hypoxic environment. This result is also similar with the report of Frezza and Bing OHL¹³⁾ and shows same tendency with Tpd, $+dp/dt_{max}$, and $-dp/dt_{max}$.

The findings of these experiments emphasize the limitations of isolated muscle studies to define the relationship between oxygenation and mechanical activity of cardiac muscle. And it is quite difficult to determine the effect of hypoxia on myocardial function independent of the other variables, such as the central nervous system, the sympathetic activities, the metabolic rate, the renal clearance, and the serum pH. Therefore we have to evaluate the effect of hypoxia step by step to clarify the effect of other variables. Further studies are needed to elucidate this important issue.

Surprisingly, this study showed obvious depression of contractility even though the oxygen concentration is higher than 21%. We always consider it safe if we do not lower the oxygen concentration below 33% during anesthesia. But, to myocardium, especially for the patient who has low cardiac reserve, is it really safe?

In summary, this study revealed that the middle-aged rats showed better tolerance to decreasing oxygen supply than the young ones, especially in Tpd, $+dp/dt_{max}$, $-dp/dt_{max}$ and $RT_{90\%}$. We could not clarify the increased susceptibility of senescent cardiac muscle to hypoxia with this experiment only. We might say that myocardial susceptibility to damage from hypoxia is greater in younger patients than aged ones even though there is progressive functional decline in cardiovascular system, as well as other organs, with getting old.

CONCLUSION

Isometric contraction study was done in the isolated rat left ventricular papillary muscles to evaluate the difference of the tolerance to relative hypoxia in the young and middle-aged rats. The middle-aged group showed better tolerance to decreasing oxygen supply than the young ones, especially in Tpd, $+dp/dt_{max}$, and $-dp/dt_{max}$. For the changes in Tr, TPT, and $RT_{90\%}$, there were no remarkable changes except of progressively decreasing $RT_{90\%}$ with decreasing oxygen concentration, which was more significant in YG.

We might say that the middle-aged rats showed better tolerance to relative hypoxia. Therefore we may have to pay more attention to young patients whose ages are over infant

when they were exposed to hypoxia.

REFERENCES

- 1) Gennser G. *Influence of hypoxia and glucose on contractility of papillary muscles from adult and neonatal rabbits.* *Biol Neonate* 1972; 21: 90-106.
- 2) Fazekas JF, Alexander FAD, Himwich HE. *Tolerance of the newborn to anoxia.* *Am J Physiol* 1941; 134: 281-287.
- 3) Heller LJ, Whitehorn WV. *Age-associated alterations in myocardial contractile properties.* *Am J Physiol* 1972; 222: 1613-1619.
- 4) Weissler AN, Kruger FA, Baba N, et al. *Role of anaerobic metabolism in the preservation of functional capacity and structure of anoxic myocardium.* *J Clin Invest* 1968; 47: 403-416.
- 5) Brooks WW, Ekblom B, Bing OHL. *Comparative response of 2-week and 6-month old rat myocardium to hypoxia.* *J Dev Physiol* 1985; 7: 229-240.
- 6) Dhalla NS, Yates JC, Walz DA, et al. *Correlation between changes in the endogenous energy stores and myocardial function due to hypoxia in the isolated perfused rat heart.* *Can J Physiol Pharmacol* 1972; 50: 333-345.
- 7) Gelli MG, Enhörning G, Hultman E, et al. *Glucose infusion in the pregnant rabbit and its effect on glycogen content and activity of foetal heart under anoxia.* *Acta Paediat Scand* 1968; 57: 209-214.
- 8) Su JY, Friedman WF. *Comparison of the responses of fetal and adult cardiac muscle to hypoxia.* *Am J Physiol* 1970; 218: 1780-1788.
- 9) Bing OHL, Brooks WW, Inamdar AN, et al. *Tolerance of isolated heart muscle to hypoxia: turtles rats.* *Am J Physiol* 1972; 23: 1481-1485.
- 10) Paradise FP, Schmitter JL, Sumritis JM. *Criteria for adequate oxygenation of isometric kitten papillary muscle.* *Am J Physiol* 1981; 241: H348-H353.
- 11) Fisher VJ, Lee FJ, Marlon AM, et al. *Paired electrical stimulation and the maximal contractile response of the ventricle.* *Circulation Res* 1967; 20: 520-533.
- 12) Frezza WA, Bing OHL. *PO_2 -modulated performance of cardiac muscle.* *Am J Physiol* 1976; 231: 1620-1624.