Effect of Aging on Ability to Acclimate to Chronic Hypoxia of Simulated High Altitude[1]

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Abstract

Young (1 month old) and old adult (13 months old) rats were subjected to a simulated high altitude (23,000 feet for 5 weeks) environment. The anoxic resistance of isolated right ventricles, right ventricular weights and hematocrit values were then measured in both sea level control and altitude-acclimated animals from both age groups. This research demonstrates that the hematocrit cannot be increased in older individuals to such an extent as in young individuals and that the right ventricular hypertrophic response to hypoxis is markedly decreased in the older animal. Older animals were shown to have increased their myocardial anaerobic competency as a result of the aging process. Young animals can be forced to increase their anaerobic competency by chronic exposure to hypoxia, but the old myocardium cannot be forced to induce further anaerobic competency by such hypoxia exposure.

Introduction

It has been well established that several parameters of the cardiovascular system are modified as a result of chronic exposure to the hypoxia of high altitude (9, 11, 16, 17, 29). Increased hematocrit, or polycythemia, has been shown to be a meaningful high altitude adaptation by several researchers, both on the basis of increasing the oxygen-carrying capacity of the blood (8, 11, 29) and of increasing the capacity of the animal to perform work under hypoxic conditions (7, 21). This enhanced erythropoietic activity has been shown to be mediated by a hormonal factor which produces bone marrow hyperplasia accompanied by an augmented rate of iron turnover (18, 19).

Right ventricular hypertrophy has been shown to consistently accompany high altitude exposure, whether one employs laboratory animals acclimated to simulated high altitude or natively acclimatized high altitude species (9, 10, 31, 32). This right ventricular hypertrophy is reflected in the EKG as a right-axis deviation (20), and it has been shown to result from the pulmonary hypertension which accompanies the hypoxia of high altitude acclimation (1, 9, 20, 27.)

In addition to these anatomical changes which accompany altitude acclimation, several investigators have demonstrated an increased

anaerobic capacity of the brain (2), straited muscle (2, 13, 17) and cardiac muscle (14, 15, 16, 17, 22), as well as an increased percentage recovery of cardiac muscle after an anoxic stress (23).

By examining these three parameters—hematocrit, heart weight and myocardial anoxic resistance—in sea level rats and in altitude-acclimated rats whose ages ranged from young to old adult it would be possible to show whether or not the aging process had altered the animal's ability to develop these cardiovascular adaptations in response to chronic hypoxia.

Materials and Methods

Animals

Male albino rats (Sprague-Dawley) were purchased from Purina Laboratory Animals, Vincentown, N. J. These rats were fed a special thyroxine-free synthetic diet (Vitamin B complex test diet, with salt mixture USP XIV substituted for Salt Mixture #2, completely vitamin and mineral fortified and pelleted for rats) purchased from Nutritional Biochemicals and tap water *ad libitum*. The animals were maintained on this test diet for 2 weeks before experimentation was begun, and this diet was administered for the duration of the experiment. Animals employed in the study were either 1 month or 13 months old. These represent respectively young and older mature rats.

Lighting, Temperature and Altitude

Animals were maintained in the laboratory at 25±1°C, and the fluorescent lighting provided 12 hours of light and 12 hours of darkness daily. The altitude-exposed animals were maintained in altitude chambers at a simulated 23,000 feet above sea level (14, 15). The high-altitude animals were acclimated for 2 days at 7,000 feet, 3 days at 10,000 feet, 3 days at 17,500 feet, 3 days at 20,000 feet, and for the remaining 5 weeks at 23,000 feet. These altitude-exposed animals remained at 23,000 feet for 72-hour intervals; they were then returned to sea level for 1 hour to allow for cleaning, disinfecting and feeding and were then depressurized to 23,000 feet for another 72-hour interval. This depressurization was accomplished gradually over an hour period so as to avoid aeroembolism. The "sea level controls" were maintained in the same laboratory at a Philadelphia, Pennsylvania, altitude of approximately 150 feet above sea level.

Hematocrit Determinations

The animals were anesthetized with ether, guillotined, and heparinized micro-hematocrit tubes were filled with blood from the severed carotids and were spun down on a clinical centrifuge to determine each animal's hematocrit value.

Right Ventricular Function Test

After blood was collected for hematocrit determinations, the rat was restrained on a dissecting board, an incision was made from the

ventral mid-line of the neck to the xyphoid process, and the ribs and musculature on the left side of the sternum were severed and separated with a wound retractor. The pulsating heart was removed and placed in a beaker of well-aerated (95% O_2 - 5% CO_2) Krebs-Ringer's bicarbonate solution to remove the excess blood. The right ventricle was dissected away (Fig. 1) and was rapidly transferred into the Krebs-Ringer's bicarbonate-filled muscle chamber (Fig. 2) which was aerated by the 95% O_2 - 5% CO_2 mixture. After an initial stabilization period of 30 minutes, the aerating mixture was changed to 95% N_2 - 5% CO_2 as an anoxic test. This anoxic test was applied for a 20-min period, and then the 95% O_2-5% CO_2 mixture was reintroduced for a 20-min recovery period. During the entire test the isolated right ventricle was kept at 29° C and was driven at a supramaximal stimulus strength (10 volts, 7 msec) at a frequency of one stimulus per second. The apical end of the excised ventricle (Fig. 1) was pinned and attached to an isometric strain gauge (Stratham Instruments). The output signal from the strain gauge was recorded on an Electronics for Medicine recording system, and the resting tesion of the excised ventricle was set a 1 g.
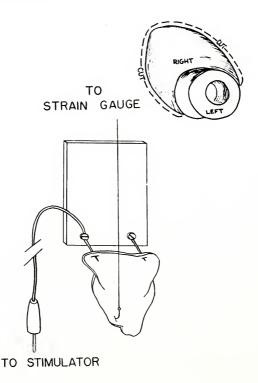

FIGURE 1. *Right ventricular procurement and fixation to muscle chamber and strain gauge.*
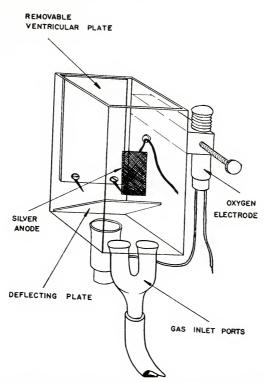
FIGURE 2. *Plexiglass muscle chamber used to conduct anoxic resistance tests.*

Heart Weight

At the conclusion of each experiment, the excised right ventricle was blotted and weighed on a Mettler H 20 T (Fisher Scientific) and was analyzed on the basis of 100 g of body weight.

Results

Hearts were analyzed on the basis of right ventricular weight per 100 g of body weight. Right ventricular weights were increased in both young and old adult rats by altitude exposure (Fig. 3). However, while altitude acclimation induces a 171% increase in right ventricular weight in the young (2 months of age) group of rats, the same hypoxic exposure elicits only a 69% increase of the same parameter in the older (14 months of age) group.

Hematocrit values were determined and compared among both age groups at sea level and after altitude exposure (Fig. 4). When compared to sea level controls of the same age, altitude acclimation elicits a 92% increase in the hematocrit value of the young group, but the older group shows only a 67% increase following the same chronic hypoxic exposure. The maximal hematocrit values achieved by the two groups
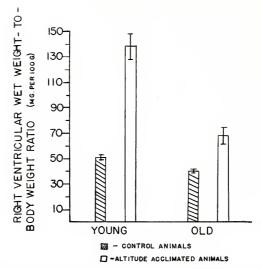
FIGURE 3. *Relationship between ventricular weight and ambient pO_2 in young and older mature rats. Vertical line indicates mean $\pm$ 1 S. D.*

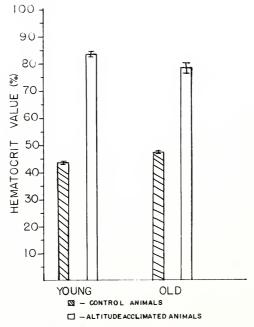after altitude acclimation, however, are not significantly different $(P > 0.05)$.

FIGURE 4. *Hematocrit changes in young and old rats in response to chronic hypoxia. Vertical line indicates mean $\pm$ 1 S.D.*

TABLE 1. *Effect of aging on certain cardiovascular adaptations to chronic hypoxia of high altitude.*

Age Group	Treatment	n	Hematocrit	S.E.	Right Ventricular Weight (mg/100 g Body Wt.)	S.E.
Old Adult (14 mo.)	Sea-level Control	13	46.9	0.6	40.0	1.5
Old Adult (14 mo.)	23,000 ft.-5 wks.	6	78.5	1.7	67.9	6.4
Young (2 mo.)	Sea-level Control	11	43.2	0.4	51.2	1.8
Young (2 mo.)	23,000 ft.-5 wks.	10	82.9	1.2	138.5	10.2

Percentage Preanoxic Contractility Remaining After

Age Group	120 anoxic contractions (2 min)	n	S.E.	480 anoxic contractions (8 min)	S.E.	960 recovery contractions (16 min)	S.E.
Old Adult (S.L.C.) (14 mo.)	79.5	13	2.7	25.1	3.5	26.4	1.7
Old Adult (Alt.) (14 mo.)	79.6	6	4.1	24.9	5.9	38.5	5.1
Young (2 mo.) (S.I.C.)	69.0	11	2.2	7.7	0.9	35.3	1.3
Young (2 mo.) (Alt.)	67.3	10	2.4	25.0	1.5	43.0	4.4

To compare both early and late responses to anoxia, contractile strengths were measured after both 120 and 480 anoxic contractions and were expressed as a percentage of the control preanoxic strength (Table 1); after the 20-min anoxic period had elapsed, the gassing mixture was switched back to 95% O_2 - 5% CO_2, and the recovery was measured after 960 additional contractions in the aerobic recovery period. The recovery was then expressed as a percentage of the control preanoxic strength (Table 1). It can be seen that whereas altitude acclimation afforded the young animals no increase in preanoxic contractility after 2 min (120 anoxic contractions) when compared to young sea level controls and a 225% increase in the same parameter after 8 min into the anoxic period (480 contractions), the old altitude-acclimated animals showed no increase in anoxic resistance when compared to old sea level controls after either 2 min or 8 min. On the other hand, when compared to young controls, the old controls showed a 15% added anoxic resistance after 2 min into anoxia and a 226% added anoxic resistance after 8 min of anoxia. Thus, while old sea level-control rats show a greater anoxic resistance than do young sea level-control animals, the anaerobic competency of the young ventricle can be increased to the same level as that of the old ventricle by chronic altitude acclimation. The older ventricle, however, cannot be forced to increase its anaerobic competency by altitude exposure.

Both age groups were compared after 16 minutes of recovery with the 95% O_2 - 5% CO_2 mixture (Table 1). While the young and old acclimated animals and the young sea level control animals have recovered 43%, 39% and, respectively, of their preanoxic contractility values, the old sea level control animals have regained only 26% of their original control value.

Discussion

While our results indicate that older animals do not show as great a percentage change in their hematocrit as do young animals in response to the same hypoxic stress, both age groups were able to reach the same maximal hematocrit value in response to the hypoxic stress. This finding contrasts with a previous study on humans by Dill (5, 6), in which he reports that both the rate and equilibrium value of hematopoiesis during altitude exposure may be depressed by the aging process. These studies, however, involved only a few subjects who were tested after only a 2-week sojourn at 3,000 m. Our animals, on the other hand, remained at 7,012 m for 5 weeks.

The fact that the older right ventricle does not show the extent of hypertrophy as does the young right ventricle in response to the hypoxia of chronic altitude exposure may be due to several factors. It has been established that the right ventricular hypertrophy of the altitude-acclimated animal results from pulmonary hypertension which accompanies exposure to hypoxia (1, 9, 20, 27). This implies, however, that: 1) the lung vasculature must be reactive to the hypoxia to which it is exposed, and 2) the right ventricle is capable of responding to this extra work load by increasing its muscle mass. The first of the require-

ments is diminished by the aging process, for active microvascular responses are both more limited and slower in older animals (3, 33). The second requirement is modified by the facts that in the aging muscle there is a decrease in the concentration of DNA and RNA, there is a decreased uptake of amino acids by the older muscle cell and there is a decrease in essential transaminases required for protein synthetic reactions (3). This decreased ability of the aging myocardium to respond to greater work loads by hypertrophy has been noted in the dialated ventricles of old rats (28). In addition, Poupa postulates that while young animals increase their cardiac muscle mass in response to increased work loads primarily by increasing the *number* of myocardial cells (hyperplasia), older animals respond to the added work load by increasing the *size* of pre-existing myocardial cells (24, 26). Furthermore, according to many researchers, there is an actual disappearance of patent vasculature in the myocardium of the old animal with aging (24, 25, 26, 30). As Poupa points out, "In young animals, overloading of the heart leads to an accelerated growth of both structures (cardiac cells and capillaries), while in adult animals growth is confined to cardiac cells only" (24).

Thus, in comparison with the younger myocardium during hypoxic exposure, the aging heart may be given less of an increased work load as a result of less reactive pulmonary vasculature, the actual protein synthetic reactions required to effect an increase in ventricular mass are depressed by aging, and the older myocardium cannot increase the oxygen-supplying structures to the muscle cells while increasing the oxygen-consuming structures as can the young myocardium. All of these aging changes may contribute to the fact that the older myocardium responds with far less increase in muscle mass than does the young myocardium to a given hypoxic stress.

Finally, this research indicates that while the aging myocardium possesses a greater anaerobic competency than does the young myocardium, the anoxic resistance of the older myocardium cannot be increased by exposure to chronic hypoxia while that of the young myocardium is greatly increased. This finding supports a recent observation of McGrath *et al.* (unpublished, personal communication) that the older myocardium cannot be forced to increase its anaerobic competency by chronic hypoxic exposure. What might be responsible for the increased anerobic competency in the older sea level-control animal, and why could it be increased no further by hypoxic acclimation? It has been demonstrated that the oxidative metabolism of the myocardium decreases greatly with age with little or no deterioration of the glycolytic and dehydrogenating activity (3). Since ATP levels are maintained at the normal level in the myocardium of the aging animal and since the ATP-ase activity of the myosin does not change with age (3), the demand of ATP for myocardial contraction in senility must remain unchanged. Since myocardial aerobic metabolism has decreased with aging, some alternative anaerobic metabolic mechanism must be employed to a greater degree if energy requirements are to be met—*i.e.*,

if some enzymatic oxidative activities are decreased by the aging process, others must be increased compensatorily if energy requirements remain constant and are satisfied (3). Such a mechanism has been elucidated in the aging erythrocyte, such that as its aerobic competency decreases with age, its anaerobic competency increases so that the concentration of ATP remains constant (3). The search for such a mechanism in the aging myocardial cell continues, however, for the pentose phosphate pathway appears to remain relatively unchanged (3) and the total LDH activity reaches its peak and then begins to decrease at 30 weeks of age as do the H_4 end M_6 isoenzymes (12).

If the myocardial cell in the older animal is employing its anerobic processes to a greater degree than is the young myocardial cell in compensation for a decreased aerobic capacity, one should expect to see evidence of a heightened anaerobic capacity in the older cell. Indeed, our results indicate the presence of such an increased anaerobic competency as added anoxic resistence in the older myocardium. Also, since the diffusion distance for oxygen from the coronary capillaries to the myocardial cell is increased in the aging ventricle due to hypertrophy of the myocardial cells and to the disappearance of patent capillaries (24, 25, 26, 30), local cellular hypoxia may also have further induced the development of a greater anaerobic competence in the older heart (4). From the results of this experiment, it would appear that the young myocardium can be influenced by chronic hypoxia to increase its anaerobic competence to the maximal level at which the older sea level controls are already operating. Under these experimental conditions, the old adult cannot be stimulated to increase its anaerobic capacity above this maximal level.

Acknowledgements

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