PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 177, 308–311 (1984)

Strain and Sex Differences in the Cardiopulmonary Adaptation of Rats to High Altitude (41948)

L. C. OU AND R. P. SMITH

Department of Physiology and Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire 03756

Abstract. On chronic exposure to hypoxia, the commercially available Hilltop (H) strain of male Sprague–Dawley rats develops severe pulmonary hypertension, right ventricular hypertrophy (RVH), and polycythemia. These signs of chronic mountain sickness are associated with a high mortality rate. In contrast, the Madison (M) strain of Sprague–Dawley rats remains healthy with significantly less severe cardiopulmonary and hematological responses. Breeding experiments under locally controlled conditions were undertaken to determine if the differences between the two strains were genetically determined and to look for possible sex differences. Following 30 to 50 days exposure to a simulated altitude of 18,000 ft, the first generation of male H rats exhibited a higher right ventricular peak systolic pressure (RVPP), a more pronounced RVH, and a greater degree of polycythemia than the male M rats. The H rats had a mortality rate of 40% in contrast to a rate of 0% in the male M rats. The first generation of female H rats also developed a higher RVPP, a greater RVH, and more severe polycythemia than that in the female M rats. There were no differences in RVPP or RVH between the males and females of either strain. Females of both strains tolerated the hypoxic exposure with a 0% mortality rate. The data suggest that the differences between the males of H and M strains in their cardiopulmonary and hematological responses and in their susceptibilities to chronic hypoxia are genetic in nature. They further suggest that the female resistance to hypoxia is not due to milder cardiopulmonary responses. Perhaps female rats tolerate RVH better than male rats, at least of the H strain.

Male Sprague–Dawley rats from two different animal suppliers exhibited strikingly different susceptibilities and cardiopulmonary responses to chronic hypoxia (1). During 30 to 40 days of exposure to a simulated altitude of 18,000 ft, Hilltop (H) rats developed severe pulmonary hypertension (PH), right ventricular hypertrophy (RVH), and polycythemia. Seventy percent of these hypoxic animals died with signs of right ventricular failure (1). In contrast, all Madison (M) rats survived identical conditions of hypoxia, appeared healthy, and exhibited lesser degrees of PH, RVH, and polycythemia (1). The differences in the cardiopulmonary and hematological responses to chronic hypoxia between H and M rats resemble those between patients with chronic mountain sickness (CMS) and healthy natives at high altitudes (2–5).

An important question arises as to whether the differences between these animals from the two suppliers are intrinsic in nature, or simply due to environmental factors such as diet. Breeding experiments, were, therefore, undertaken to address this question. As a result of the breeding experiments, an opportunity was also provided to examine the influence of sex on the different susceptibilities and cardiopulmonary responses to chronic hypoxia between the two groups.

Materials and Methods. Male and female Sprague–Dawley rats were obtained from two commercial suppliers, Hilltop (H, Scottdale, Pa.) and Madison (M, Madison, Wisc.). Females weighed from 180 to 200 g and males from 270 to 290 g. Providing females that were smaller than the males was of particular importance in achieving pregnancy among the H rats. The locally bred male and female pups were raised under identical conditions, separated at the age of 21 days, and exposed to a simulated altitude of 18,000 ft (5500 m) at about 8 weeks of age. The body weights ranged from 260 to 290 g for the males and 220 to 250 g for the females in both groups. At the 30th and 50th days of exposure, five to eight animals each of the males and females from both the H and M groups were studied for their cardiopulmonary and hematological changes as described previously (1). Briefly,

0037-9727/84 $1.50

right ventricular peak pressure (RVPP), as a measure of systolic pulmonary arterial blood pressure, was determined in rats anesthetized with a combination of ketamine (60 mg/kg body wt, im) and pentobarbital (20 mg/kg body wt, ip), with body temperature maintained at 37.0 ± 1.0°C by means of a heating pad. The right external jugular vein was catheterized. The catheter was connected via a Statham transducer to a Grass oscillograph for blood pressure recording. The catheter was then advanced until the RVPP was obtainable. We have previously shown the RVPP as measured under anesthesia was virtually the same as in fully awake rats (1). The animal was then sacrificed by exsanguination. The whole heart was removed and placed in 4% Formalin in physiological saline for 3 days. The right and left ventricles and the septum were then dissected from the heart according to Fulton *et al.* (6). Hematocrits were measured by a microtechnique.

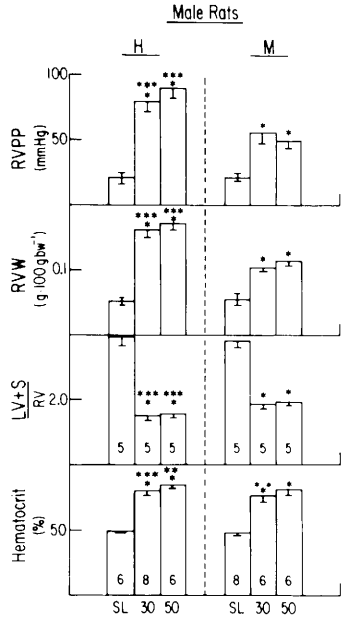

FIG. 1. Cardiopulmonary and hematological responses to chronic exposure to a simulated altitude of 18,000 ft in male H and M strains of rats. The numerals 30 and 50 denote days at high altitude and SL indicates sea level controls. Bar denotes means ± SD. Figure within bars denotes number of animals studied. *P values < 0.05 in comparison to sea level controls within the same panel. **P values < 0.05 in comparison to the other exposed group of the same panel. ***P value < 0.05 in comparison to the other strain for the same duration of exposure. Controls were studied after 30 days at SL pressure.

TABLE I. STRAIN AND SEX DIFFERENCES IN MORTALITY DURING 50-DAY EXPOSURE TO A SIMULATED ALTITUDE OF 18,000 FT

Strain:	Hilltop (H)		Madison (M)	
Sex:	M	F	M	F
Number alive	14	24	12	12
Number dead	9	0	0	0
P^a		0.01	0.02	—

[a] P values for comparison with males of the H strain by χ^2 analysis.

Statistics. The data were analyzed either by Student's t test or by variance analysis, as appropriate. Multiple comparisons were analyzed by the Newman–Keuls test (7). Differences are reported as statistically significant when $P < 0.05$.

Results. Figure 1 summarizes the cardiopulmonary and hematological changes following 30 and 50 days of hypoxic exposure in both the H and M strains of male rats. Thirty days of hypoxia greatly increased the RVPP, the degree of RVH (whether expressed as increased RV to body weight ratio or decreased LV plus S to RV weight ratio), and the Hct in the H rats. Prolonging the exposure from 30 to 50 days did not further change the RVPP or the degree of the RVH, but it did slightly increase the HCT in H rats ($P < .05$) (left panel, Fig. 1). In the male M rats, hypoxic exposure of both 30- and 50-day duration resulted in consistently less severe changes in these cardiopulmonary and hematological parameters. Like the response in the H rats, prolonged hypoxic exposure further increased the Hct ($P < 0.02$) in the M rats. The sea level control values for RVPP, RV to body weight ratio, LV plus S to RV weight ratio, and the Hct were similar in the two strains. Nine out of twenty-three (39%) of the locally bred male H rats died whereas all locally bred M rats survived and appeared healthy (Table I).

The cardiopulmonary and hematological responses to hypoxia of H and M strains of female rats are presented in Fig. 2. There was no difference between the two strains in

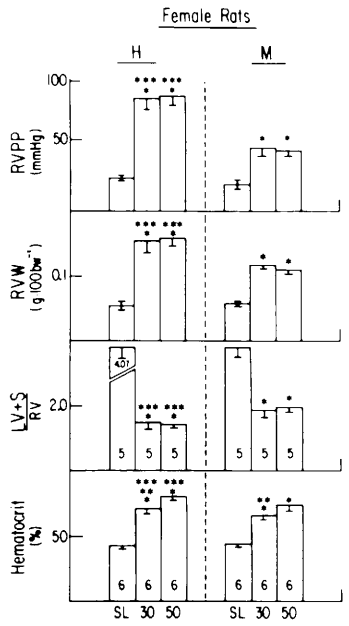

FIG. 2. Cardiopulmonary and hematological responses to chronic exposure to a simulated altitude of 18,000 ft in female H and M strains of rats. See Fig. 1 for explanation.

any of the parameters shown under SL conditions. Hypoxic exposure of 30 and 50 days increased the RVPP, degree of RVH, and the Hct to a significantly greater extent in the H than in the M female rats. Again, prolonged hypoxic exposure further increased the Hct in the females of either strain. All females irrespective of strain survived the 50 days hypoxic exposure (Table I).

Discussion. The present study clearly demonstrates that the locally bred male H and M Sprague–Dawley rats exhibited the same differences in their mortality and cardiopulmonary and hematological responses to chronic hypoxia, as those supplied directly from the corresponding breeding laboratories (1). Therefore, the differences between the H and M strains of rats in the susceptibilities and cardiopulmonary and hematological responses to chronic hypoxia are congenital and genetically determined characteristics. The findings from the locally bred females of the two strains further support this conclusion.

The observation that the females were more resistant to chronic hypoxia than the males (Table I) is in agreement with the experience with humans (2, 5), cattle (8), swine (9), chickens (10), and rats (11, 12). The cause for the sex difference, however, has not been firmly established. It has been shown that susceptibility to chronic hypoxia may relate to a cardiopulmonary system that is more reactive to hypoxia as evidenced by a more pronounced RVH (9–11) and a more severe pulmonary hypertension (12) in the males than in the females. In contrast, the present study showed that chronic hypoxia of both 30 and 50 days resulted in similar changes in the RVPP and the degree of RVH in the males and in the females of both the H and M strains. The mean pulmonary arterial blood pressure (PAP) in awake animals was measured in the studies of Rabinovitch *et al.* (12) and of McMurtry *et al.* (9) whereas the RVPP in the anesthetized animals was measured in the present study. This may conceivably contribute to the discrepancy. On the other hand, the RVPP is equal to the systolic PAP, and the RVH results from, and is closely correlated with, the pulmonary hypertension under chronic hypoxia. The absence of sex difference in both RVPP and RVH as found here should preclude any major sex differences in the cardiopulmonary responses to hypoxia, at least, under the present experimental conditions.

Our observations substantiate those of Swigart on rats (13) and of Sillau *et al.* on chickens (14). Under conditions of hypoxic exposure similar to those of the present study, Swigart (13) found that both male and female rats developed comparable RVH. In an experiment with large numbers of chickens, Sillau *et al.* (14) observed a higher incidence of right ventricular failure in the males than in the females following chronic hypoxia despite the absence of any sex difference in the resulting pulmonary hypertension or RVH. It is perhaps relevant that the mean PAP in conscious animals was measured in the study of Sillau *et al.* (14). Similar to that are the studies of Rabinovitch *et al.* (12) and of McMurtry *et al.* (9). Thus, our results and those of Swigart (13) and of Sillau *et al.* (14) failed to demonstrate a more reactive cardiopulmonary system to chronic hypoxia in the males than in females. This suggests that factors other than a reactive cardiopulmonary

system must account for male susceptibility to chronic hypoxia. Or, the female rats simply tolerate severe pulmonary hypertension and RVH better than the male rats.

The underlying mechanisms for the different cardiopulmonary and hematological responses to hypoxia between the H and M strains of rats have not been fully elucidated. Strain differences in ventilatory (15) and pulmonary vasoconstrictive (16) responses to hypoxia have been ruled out as the primary cause. However, Hill and Ou (16) showed in isolated lungs perfused with sea level normocytic blood that chronic hypoxia resulted in a greater vascular resistance to flow in the H than M rats. This observation suggests that chronic hypoxia led to more profound structural alteration of the pulmonary vasculature in the H than the M rats, and this could, at least partly, contribute to the exaggerated cardiopulmonary response to hypoxia in the former. Following chronic hypoxia, the hematocrits were consistently higher in the male H than M rats (1, 15, 16) and this was also true for the females as shown in the present study. More recently, we found (17) that the circulating erythrocyte mass and total blood volume were much greater in the chronically hypoxic male H than M rats. A more severe polycythemia with its increased viscosity, a greater cardiac output due to a greater total blood volume, together with the more profound structural alteration of the pulmonary vasculature, could lead to an exaggerated pulmonary hypertension and RVH in the chronically hypoxic H rats.

It is concluded that differences in susceptibilities and cardiopulmonary and hematological responses to chronic hypoxia between the H and M strains of rats are most likely genetic. The female's tolerance to severe hypoxia cannot be attributed to a milder cardiopulmonary response to hypoxia.

This work is supported by Grants HL 21159 and HL 14127 from the National Institutes of Health.

1. Ou LC, Smith RP. Probable strain differences of rats in susceptibilities and cardiopulmonary responses to chronic hypoxia. Respir Physiol **53**:367–377, 1983.
2. Arias-Stella J. Chronic Mountain Sickness: Pathology and definition. In: Porter R, Knight J, eds. Cardiac and Respiratory Aspects. Ciba Foundation Symposium: High Altitude Physiology, London, Churchill, pp31–40, 1971.
3. Heath D, Williams DR. Man at High Altitude. New York, Churchill, pp169–179, 1981.
4. Monge CM, Monge CC. High Altitude Diseases: Mechanism and Management. Springfield, Ill., Thomas, pp32–47, 1966.
5. Penaloza D, Sime F, Ruiz L. Cor pulmonale in chronic mountain sickness: Present concept of Monge's disease. In: Porter R, Knight J, eds. Cardiac and Respiratory Aspects. Ciba Foundation Symposium: High Altitude Physiology, London, Churchill, pp41–52, 1971.
6. Fulton RM, Hutchinson EC, Jones AM. Ventricular weight in cardiac hypertrophy. Brit Heart J **14**:413–420, 1952.
7. Snedecor GW, Cochran WG. Statistical Methods. Ames, Iowa State Univ Press, 7th ed, 1981.
8. Blake JT. Occurrence and Distribution of Brisket Disease in Utah. Utah Agric Exp Stn Circular 151, 1968.
9. McMurtry IF, Frith CH, Will DH. Cardiopulmonary responses of male and female swine to simulated high altitudes. J Appl Physiol **35**:459–462, 1973.
10. Burton RR, Besch EL, Smith AH. Effect of chronic hypoxia on the pulmonary arterial blood pressure of the chicken. Amer J Physiol **214**:1422–1438, 1968.
11. Smith P, Moosavi H, Winson M, Heath D. The influence of age and sex on the response of the right ventricle, pulmonary vasculature and carotid bodies to hypoxia in rats. J Pathol (London) **112**:11–18, 1974.
12. Rabinovitch M, Gamble WJ, Miettinen OS, Reid L. Age and sex influence on pulmonary hypertension of chronic hypoxia and on recovery. Amer J Physiol **242**(Heart Circ Physiol **9**):H62–H72, 1981.
13. Swigart RH. Polycythemia and right ventricular hypertrophy. Circ Res **17**:30–38, 1965.
14. Sillau AH, Cueva S, Morales P. Pulmonary arterial hypertension in male and female chickens at 3300 m. Pfluegers Arch (Eur J Physiol) **386**:269–275, 1980.
15. Ou LC, Hill NS, Tenney SM. Ventilatory responses and blood gases in susceptible and resistant rats to high altitude. Respir Physiol, in press.
16. Hill NS, Ou LC. The role of pulmonary vascular responses to chronic hypoxia in the development of chronic mountain sickness in rats. Respir Physiol, in press.
17. Cai YN, Ou LC, Smith PR. Severe polycythemia and hypervolemia in a rat model of chronic mountain sickness. Fed Proc **43**:905, 1984.

Received January 24, 1984. P.S.E.B.M. 1984, Vol. 177.
Accepted June 29, 1984.