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Effects of Sex Hormones on Cardiovascular and Hematologic Responses to Chronic Hypoxia in Rats (40268)

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Males seem more susceptible than females to diseases marked by hypoxia and right heart failure such as chronic obstructive lung disease, chronic mountain polycythemia, and sleep apnea (1-4). At high altitude, decreased pulmonary hypertension, less right ventricular hypertrophy, and higher arterial oxygen tensions are observed in female compared to male swine (5). Higher arterial oxygen tensions are also reported for human female compared to male newcomers at high altitude (6). Sex hormones are known to influence variables affecting oxygenation. Progesterone is a ventilatory stimulant at low and, especially, at high altitude (7, 8). Estrogen reduces while progesterone and testosterone enhance the amount of aortic wall thickening during systemic hypertension (9, 10). Testosterone stimulates erythropoiesis, augments 2,3-diphosphoglycerate (DPG) production and shifts the oxygen dissociation curve rightward (11, 12).

The purpose of this study was to examine the effects of estrogen, progesterone and testosterone on right ventricular hypertrophy, erythropoiesis, red cell 2,3-DPG production and oxygen dissociation curve position in rats during high altitude exposure. We hypothesized that the female sex hormones would protect against right ventricular hypertrophy whereas the male hormone might aggravate its development. Testosterone might also have beneficial effects through shifting the oxygen dissociation curve rightward, thereby increasing tissue oxygen delivery.

Materials and methods. Sixty-four male, 300 g, Sprague-Dawley rats were castrated and allowed to recover for 2 weeks prior to treatment. Half of the rats were maintained at the Denver laboratory altitude of 1520 m, and half were exposed in a hypobaric chamber to a simulated high altitude of 4000 m. At each altitude, rats were assigned randomly to groups of eight which received weekly intramuscular injections of either saline, estrogen (Depo-Estradiol Cypionate, 1 mg/ml), progesterone (Depo-Provera, 100 mg/ml), or testosterone (Depo-Testosterone Cypionate, 50 mg/ml). The saline groups were used to control for the effects of hormone treatment; the four groups residing at 1520 m served as controls for the effects of high altitude. The hormones used were long-acting preparations supplied by Upjohn (Kalamazoo, Michigan). Progesterone and testosterone were each given in doses of 5 mg weekly to each animal. Estrogen was given in a dose of 20 μg weekly to each animal. These levels were chosen as pharmacologic dosages to assure response and are in accord with other studies in rats using the same hormone preparations (9, 10, 12, 13). Body weights were measured weekly.

After 5-6 weeks of exposure to their respective altitude and hormone treatments, the rats were anesthetized with sodium pentobarbital (30-40 mg/kg body wt, ip), tracheostomized and ventilated with a rodent respirator (Harvard Apparatus, Model 680). The carotid artery was exposed and catheterized with PE-50 tubing (Clay Adams) to record systemic arterial pressure and collect arterial blood samples. A Statham P23Db pressure transducer was used to record pressure on an Electronics For Medicine recorder after calibration with a mercury manometer. An arterial blood sample was taken immediately after catheterization for measuring hematocrit with the capillary tube method. Continuous recordings of carotid pressure were made during normoxia (room air or 30% O_2 in N_2). Blood (10-12 ml) was then drawn for measuring hemoglobin levels colorimetrically, 2-3 DPG levels enzymatically (14), and the position of the oxygen dissociation curve as described below. Two blood samples (4 ml) were mixed in a tonometer at 38° for 10 min with humid gas mixtures so as to yield PO_2's of approximately 30 and 40 mmHg.

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Blood samples were kept anaerobic while PO_2, PCO_2, and pH were read at 38° using Radiometer microelectrodes and O_2 content was read with a Lex-O_2-Con oxygen analyzer (Lexington Instruments). PO_2 was corrected to pH = 7.400 and PCO_2 = 40 mmHg (15). O_2 capacity was measured with the Lex-O_2-Con on a blood sample equilibrated three times with 30% O_2 for the calculation of O_2 saturation. The resulting two pairs of O_2 tension and O_2 saturation measurements were used to compute the P_{50}, or the PO_2 at which hemoglobin is half-saturated with oxygen, with a computer program for solving the following equation derived from Hill (16): $(PO_2/P_{50})^n = SO_2/(1 - SO_2)$. Measured saturations were within the range for which the Hill equation is valid (17). Hearts were collected and dissected for the determination of wet and dry ventricular weight ratios (18).

Two-way analysis of variance with replicated measures was used to assess the effects of altitude and hormone treatment on each of the variables measured. Unequal cell frequencies were handled by the classic experimental design approach (19). The analysis included covariates to account for effects of variation in other variables on the dependent variable. Calculations were performed using the ANOVA subprogram of the Statistical Package for the Social Sciences (19). Data are reported as mean ± SEM and levels of statistical significance as $P < 0.05$ or $P < 0.01$.

Results. Body weights were the same in each group prior to hormone and altitude treatment ($\bar{X}$ = 342 g). Growth, as measured by body weight, was retarded by treatment with estrogen and by exposure to high altitude (Table I). Weight gain in testosterone or progesterone treated rats was not different from that of the saline groups.

Hematocrit rose after exposure to high altitude and treatment with testosterone (Table I). Estrogen administration decreased hematocrit in both the altitude groups. Progesterone treatment did not affect hematocrit at either altitude.

Hypertrophy of the right ventricle occurred at high altitude as indicated by an absolute increase in right ventricular weight, an increased ratio of right ventricular weight to total ventricular weight, and an increased ratio of right ventricular weight to body weight (data in Table I, Fig. 1). Testosterone treatment also induced right ventricular hypertrophy as evidenced by an absolute increase in right ventricular weight, an elevated right ventricular weight to total ventricular weight ratio, and an increased right ventricular weight to body weight ratio at both

TABLE I. CARDIOVASCULAR AND HEMATOLOGIC EFFECTS OF SEX HORMONE AND ALTITUDE TREATMENT.

Hormone	Altitude	Body wt (g)	RV wt, wet[a] (mg)	LV + S wt, wet (mg)	$\bar{P}_{systemic}$ (mmHg)	Hct (vol %)	2,3-DPG (μmoles/g Hb)	P_{50} (mmHg)
Control	low	411 ± 15[b] (8)[c]	0.181 ± 0.009 (8)	0.701 ± 0.029 (8)	145 ± 4 (6)	46 ± 1 (8)	21.9 ± 1.0 (6)	39.7 ± 3.4 (4)
	high	386 ± 8 (8)	0.256 ± 0.017 (8)	0.674 ± 0.025 (8)	142 ± 4 (7)	60 ± 2 (8)	24.6 ± 1.0 (7)	38.9 ± 0.7 (4)
Progesterone	low	395 ± 10 (8)	0.181 ± 0.010 (8)	0.699 ± 0.032 (8)	145 ± 5 (6)	46 ± 1 (8)	12.2 ± 1.2 (6)	39.6 ± 2.0 (5)
	high	376 ± 10 (7)	0.286 ± 0.011 (7)	0.714 ± 0.021 (7)	160 ± 5 (6)	59 ± 2 (8)	24.7 ± 1.9 (6)	42.3 ± 2.7 (5)
Estrogen	low	348 ± 5 (8)	0.170 ± 0.007 (8)	0.676 ± 0.023 (8)	143 ± 3 (5)	42 ± 1 (8)	24.2 ± 0.7 (5)	38.1 ± 0.3 (2)
	high	313 ± 14 (8)	0.269 ± 0.031 (8)	0.659 ± 0.045 (8)	139 ± 7 (5)	53 ± 4 (7)	24.8 ± 1.4 (5)	40.1 ± 1.8 (4)
Testosterone	low	427 ± 15 (8)	0.231 ± 0.006 (8)	0.809 ± 0.030 (8)	121 ± 10 (6)	49 ± 1 (6)	26.6 ± 1.8 (8)	38.7 ± 0.8 (8)
	high	374 ± 12 (7)	0.336 ± 0.030 (7)	0.700 ± 0.036 (8)	135 ± 7 (5)	61 ± 3 (6)	29.3 ± 2.6 (5)	38.3 ± 1.1 (5)
2-Way ANOVA								
Hormone effect		$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.01$	NS
Altitude effect		$P < 0.01$	$P < 0.01$	NS	NS	$P < 0.01$	$P < 0.05$	NS

[a] Abbreviations: RV = right ventricle, LV + S = left ventricle + septum.
[b] Mean ± SEM.
[c] Sample size.

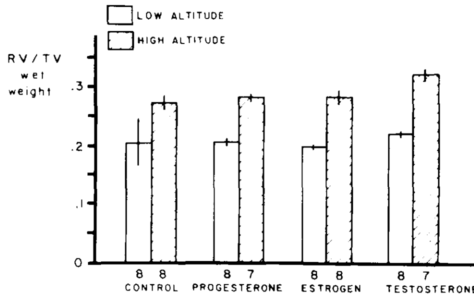

FIG. 1. Right ventricular hypertrophy, as measured by right ventricular (RV) weight to total ventricular (TV) weight, is increased at high altitude compared to low altitude ($P < 0.01$) and by treatment with testosterone compared to treatment with saline ($P < 0.01$). Data are reported as mean ± SEM. Sample sizes are shown in parentheses.

altitudes (data in Table I, Fig. 1). The right ventricular to body weight ratio was increased by estrogen treatment due to a decrease in body weight; neither absolute right ventricular weight nor the right ventricular to total ventricular weight ratio was elevated (Table I, Fig. 1). The effect of high altitude on right ventricular hypertrophy was not influenced by any of the hormone treatments, nor was the effect of hormone treatment on right ventricular hypertrophy influenced by high altitude (ANOVA probability of hormone by altitude interaction = NS). Examination of right ventricular dry weights and right ventricular to total ventricular dry weight ratios revealed that tissue water content was not altered by either altitude or hormone treatment (data not shown). The rise in hematocrit contributed to the development of right ventricular hypertrophy (analysis of covariance, $P < 0.01$) but the differences remained between altitudes ($P < 0.01$) and among hormone groups ($P < 0.01$) once the effect of hematocrit was removed. Mean systemic arterial pressure in both altitude groups was lower in the testosterone group under the conditions of measurement (Table I). The ratio of left ventricular to body weight was not depressed (Table I), suggesting that systemic pressures were not consistently lower during treatment.

2,3-DPG increased at high altitude and after testosterone treatment (Table I). However, the change in 2,3-DPG levels was not associated with differences in oxygen dissociation curve position as measured by P_{50}.

Discussion. The known metabolic effects of testosterone on protein metabolism (20) probably accounted for the higher hematocrit at both altitudes in the testosterone treated rats than in the rats treated with saline, estrogen or progesterone. However, it is not clear how the anabolic effect of testosterone could have been responsible for the selective right ventricular hypertrophy at 1520 m and at 4000 m. Increased hematocrit per se can cause a rise in pulmonary vascular resistance (21) which would contribute to greater right ventricular hypertrophy, but covariance analysis indicated that the increased hematocrit was not the sole cause.

Whatever the mechanism by which testosterone selectively increased right ventricular weight at the laboratory altitude or at high altitude, these findings have not been previously reported. The comparable percentage increases in the testosterone groups (relative to the controls) at both altitudes and the lack of significant hormone by altitude interaction suggests that the effect of testosterone was merely added to that of high altitude. A previous study has observed greater right ventricular hypertrophy at high altitude in adult male compared to adult female, juvenile male or juvenile female rats (22). These results together with the contrast between testosterone and saline treated rats suggest that testosterone was the component of maleness that was associated with greater right ventricular hypertrophy and thus probably greater right ventricular work at high altitude.

The results of this study did not support the hypothesized beneficial effect of testosterone treatment on tissue oxygen delivery. Both testosterone treatment and high altitude exposure elevated 2,3-DPG levels as has been reported (11, 12, 23), but in the present study no change occurred in oxygen dissociation curve position.

We did not find evidence to support the hypothesized protective effect of the female sex hormones against right ventricular hypertrophy. We had expected that progesterone would induce hyperventilation and thereby lessen the hypoxic stimulus for pulmonary hypertension, and that estrogen would reduce the degree of morphogenic vascular thickening (9), and, thereby, also decrease the

amount of pulmonary hypertension at high altitude. Progesterone has recently been reported not to act as a ventilatory stimulant in rats during normoxia (25), but it is not known whether the same is true during hypoxia. If true, or if castration interferes with the effect of progesterone, the present study with rats does not provide an adequate test of the effects of progesterone on hypoxic responses. Estrogen retarded body growth, slowing weight gain at 1520 m and leading to actual weight loss at 4000 m. This suggests that the dose was effective insofar as estrogen has previously been reported to reduce appetite (24). Growth retardation served to increase the ratio of right ventricular to body weight but did not affect the weight of the right ventricle alone or the ratio of right ventricular to total ventricular weight. Thus, estrogen did not induce right ventricular hypertrophy, nor did it, as hypothesized, reduce hypertrophy of the right ventricle.

In conclusion, our working hypothesis that the female hormones would protect against right ventricular hypertrophy was not sustained. The results taken together suggest that the advantage reported for the female in having less right ventricular hypertrophy at high altitude (5, 22) is not due, at least in the rat, to the presence of female hormones but rather to the absence of testosterone. In humans, where males have a particularly high incidence of diseases associated with hypoxia and right heart failure, one must consider the possibility that testosterone itself may contribute to the disease process and, in particular, the right heart failure.

Summary. Castrated male rats exposed to high altitude (4000 m) for 5–6 weeks developed right ventricular hypertrophy, increased hematocrits and increased levels of 2,3-DPG. Treatment with testosterone increased right ventricular weight, hematocrit and 2,3-DPG levels. The effects of high altitude and testosterone on right ventricular hypertrophy were additive. Estrogen treatment decreased hematocrit whereas progesterone had no effect. Neither female hormone affected 2,3-DPG levels or right ventricular hypertrophy. The results suggest that sex differences in the degree of right ventricular hypertrophy at high altitude and in the incidence of diseases marked by hypoxia and right heart failure may be more a function of disadvantages associated with the male hormone, testosterone, than with advantages associated with the female hormones, progesterone and estrogen.

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