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FOREWORD

This report was prepared in the Department of Physiology, University of Florida College of Medicine, Gainesville, Florida, by —

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ABSTRACT

Chronic exposure to an atmosphere containing 13% oxygen protects against development of renal hypertension in rats. The mechanism through which the rats are protected may involve the thyroid gland since certain criteria for assessment of thyroid function, using radioactive iodide, suggest depression of activity. Other physiologic mechanisms, brought into play as a result of hypoxia, may also contribute and need to be studied.

This technical documentary report has been reviewed and is approved.

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EFFECT OF CHRONIC EXPOSURE TO HYPOXIA ON BLOOD PRESSURE AND THYROID FUNCTION OF HYPERTENSIVE RATS

1. INTRODUCTION

The systolic blood pressure of natives of the Andes who live and work at an altitude of 10,000 to 12,000 feet (3.0 to 3.7 km.) is lower than that of individuals of similar age living at sea level (1). Incidence of cardiovascular hypertensive disease is said to be relatively rare among Andean natives. It has also been reported that natives of Ceylon living at an altitude of 6,000 feet (1.8 km.) have systolic blood pressures which average about 10 mm. Hg less than those living at sea level (2). Reasons for the lower systolic blood pressure and the lower incidence of cardiovascular hypertensive disease of residents at altitude are unknown but could be related either to physiologic, genetic, or environmental factors or to combinations of these. A study was therefore undertaken to evaluate the effect of exposure to lowered ambient oxygen tension on development of renal hypertension in genetically pure, white rats. When it was established that exposure to hypoxia prevented the rise of systolic blood pressure to the level of control rats maintained at normal oxygen tension (150 mm. Hg), the thyroid gland was singled out to determine whether it played a role. This choice was deliberate because earlier studies from this laboratory demonstrated that the thyroid gland plays an important secondary role in the development of renal hypertension in rats (3, 4).

2. METHODS

Three separate experimental series were performed. They were carried out in an

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identical manner and will, therefore, be described as a single experiment.

Thirty-six male rats of the Holtzman albino strain were used. At the beginning of the experiment the rats weighed approximately 275 gm. During a 2-week control period, systolic blood pressure was measured weekly by the microphonic manometer technic of Friedman and Freed (5) but without anesthesia. Body weight was also measured once each week. The rats were kept three to a cage in a thermoregulated room maintained at 26° C. and illuminated from 8 a.m. to 6 p.m. All rats were given tap water to drink and ground Purina laboratory chow to eat. Water was available at all times in containers of the type described by Lazarow (6). These consisted of infant nursing bottles with cast aluminum spouts. Food containers were essentially spillproof and have been described in detail recently (7).

At the end of the 2-week control period, the kidneys of all rats were bilaterally encapsulated with latex envelopes by the method of Abrams and Sobin (8). Three days were allowed for recovery from the operation after which half of the rats (18 in all) were placed in a chamber and subjected to hypoxia by the method of Sisson and Fregly (9). The remaining 18 rats were not subjected to hypoxia and served as controls for the treated group. During the first day of exposure to hypoxia, the percentage of oxygen in the chamber was reduced to 17%. Thereafter, the percentage of oxygen in the chamber was reduced by 1%per day until the rats were living in an atmosphere containing 13% oxygen. The fraction of oxygen in the chamber remained at this level until the end of the experiment,

11 weeks later. The average barometric pressure was 760.8 mm. Hg.

The chamber was opened every second day to weigh the rats, measure food and water intake, replenish the carbon dioxide (soda lime) and water vapor (concentrated sulfuric acid) absorbers, and clean the cages. The time required to make these measurements was approximately 1 hour. Hence, the rats subjected to hypoxia were returned to 21% oxygen for 1 hour out of every 48. Once each week, during the time the chamber was opened, the systolic blood pressure of all rats, hypoxia-treated and control, was measured.

At the beginning of the 10th week, hematocrit ratio, hemoglobin concentration, and red cell count were determined from blood obtained from the tails of the rats. Hematocrit ratios were measured by means of the Van Allen tube centrifuged at $2,300 \times g$ for 30 minutes. Hemoglobin concentration was measured by the cyan-methemoglobin technic of King and Gilchrist (10).

At the end of the 11th week, all rats of the first and third series were injected intraperitoneally with approximately 5 μ c. of carrier-free, radioactive iodide (NaI¹³¹). Radioactivity of the thyroid gland was determined at intervals by holding the neck of the unanesthetized rat over a scintillation probe and counting radioactivity for 1 minute. Measurements were made until successive counts of radioactivity agreed within 3%. Urine was collected from each rat for the first and second 24-hour period after injection of radioactive iodide. The radioactivity of the urine was measured in a well-type scintillation detector.

The rats of the first series were killed by exsanguination 50 hours after injection of radioactive iodide, while those of the third series were killed 105 hours after administration of radioactive iodide. Radioactivity of 1 ml. of serum and of a 10% trichloroacetic acid filtrate of 1 ml. of serum was measured in a well-type scintillation detector. From these values, the conversion ratio (radioactivity of precipitate to radioactivity of plasma) was calculated. The conversion ratio has been used as an estimate of the level of circulating thyroxine. At death, the thyroid gland was excised, cleaned of connective tissue, and weighed on a torsion balance. It was then placed in buffered 10% formalin and the radioactivity measured in a well-type scintillation detector. From the measurements mentioned above, the thyroid to serum ratio (counts/ minute/thyroid gland to counts/minute/milliliter of serum) could be calculated. This measurement is often used to estimate the concentrating ability of the thyroid gland (12).

In addition to these measurements, serum sodium, potassium, and chloride and cholesterol concentrations were measured. Serum sodium and potassium concentrations were determined by means of a lithium internal standard flame photometer. Serum chloride was measured by the method of Cotlove et al. (13), while serum cholesterol concentration was measured by the method of Pearson et al. (14).

Other organs in addition to the thyroid gland were removed at death. These include heart, kidney, testis, adrenal, thymus, seminal vesicle, and prostate. Each organ was dissected carefully of fat and connective tissue and weighed on a torsion balance.

Statistical analyses of the data were made using the t-test for the 95% confidence limit (15).

3. RESULTS

The effects of chronic exposure to an atmosphere containing 13% oxygen upon systolic blood pressure and body weight are shown in figure 1. At the end of the 2-week control period the kidneys of all rats were encapsulated with latex envelopes. Eighteen rats were subjected to hypoxia and 18 were used as controls for the treated group. Mean systolic blood pressure of control rats rose to a stable, maximal level about 7 weeks after kidney encapsulation (fig. 1A). The group exposed to hypoxia showed an elevation of systolic blood pressure which paralleled that of controls until 6 weeks







B. Mean body weight of the rats shown in A during the course of the experiment.

after kidney encapsulation. Thereafter, systolic blood pressure fell until blood pressure had reached a level of about 144 mm. Hg at the end of the experiment.

Mean body weights of the two groups for each week throughout the experiment are shown in figure iB. Control rats gained weight rapidly at first and then more slowly to reach a body weight of approximately 450 gm. by the end of the experiment. Rats exposed to hypoxia grew at approximately a linear rate so that they reached a body weight of approximately 420 gm. by the end of the experiment.

Mean water and food intakes of the two rat groups during each week of the experiment are shown in figure 2. Mean water intake (milliliters/100 gm. body weight/day) of the two groups differed during the first 2 weeks of the experiment but were similar thereafter. Mean food intake of the two groups differed





Mean water intakes of control (solid line) and hypoxia-treated rats (dotted line) are shown during each week of the experiment (upper part of the figure). Mean food intakes of the same two groups during each week of the experiment are shown in the lower part of the figure.

during the first 3 weeks of the experiment but were also similar thereafter. From the 5th week onward food intake of the rats subjected to hypoxia was slightly, but not significantly, greater than that of controls.

At the beginning of the 10th week, hematocrit ratio, hemoglobin concentration, and red blood cell count were all significantly (P < .05) greater in the rats exposed to hypoxia than in their controls (table I).

At the end of the 11th week, the uptake

and rate of release of radioactive iodide from the thyroid gland were determined. The first measurements of radioactivity were made 20 hours after injection of the radioactive iodide and showed a significant reduction in accumulation of iodide by the thyroid glands of hypoxia-treated rats (fig. 3). The rate of loss of radioactive iodide from the thyroid glands of hypoxia-treated rats, while appearing only slightly greater, is actually significantly greater than that of controls at any time during the experiment. It should be pointed out that the data up to 50 hours are means for the

TABLE I

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Group	HCT ratio	Hb conc. (gm. %)	RBC count × 10 ⁶	Serum Na conc. (mEq./liter)	Serum K conc. (mEq./liter)	Serum Cl conc. (mEq./liter)	Serum cholesterol conc. (mg./100 ml.)
Control	$43.8 \pm 1.9^*$	16.0 ± 0.5	7.2 ± 0.3	142.4 ± 0.7	6.56 ± 0.28	101.9 ± 2.0	107.9 ± 3.7

(12)

 140.1 ± 1.3

(10)

(12)

 6.74 ± 0.37

(8)

(6)

 100.1 ± 0.8

(6)

(6)

 10.8 ± 0.61

(5)

Effect of chronic exposure to an atmosphere containing 13% oxygen

*One standard error of mean.

†Number of rats.

Hypoxia

 \pm Significantly different from control (P < .05).

(12)†

 53.5 ± 1.41

(10)

(5)

 18.4 ± 0.1

(5)



FIGURE 3

Radioactivity of the thyroid gland of control (solid line) and hypoxia-treated (dotted line) rate during 105 hours after injection of radioactive iodide. One standard error is set off at each mean. Numbers in parentheses represent number of rats.

12 control and 11 hypoxia-treated rats of the first and third series combined. The data shown for all times after 50 hours are for the 6 control and 6 hypoxia-treated rats of the third series only.

Urinary excretion of radioactive iodide by each group of rats at 24 and 48 hours after injection is shown in table II. Although hypoxia-treated rats tended to excrete greater amounts of radioactive iodide during 24 and

5

(10)

 $125.1 \pm 5.3 \ddagger$

(8)

TABLE II

Effect of chronic exposure to hypoxia on urinary excretion of radioactive iodide (percent of injected dose)

Group'	. 0-24 hours	24-48 hours	0-48 hours
Control (12)*	$56.8 \pm 2.5 \dagger$	8.9 ± 0.8	65.7 ± 2.3
Hypoxia (11)	59.8 ± 3.8	10.3 ± 1.5	70.1 ± 3.7

*Number of rats.

†One standard error of mean.

48 hours after injection, differences from control values were not significant.

At the end of the measurement of release of radioactive iodide from the thyroid gland (50 hours for the rats of series 1 and 105 hours for rats of series 3), all rats were killed. Data from each series are listed separately in table III. Ratio of thyroid weight to body weight was not different for the two groups of series 1; however, acinar cell height of the thyroid gland, measured with ocular micrometer and microscope under high, dry (480 \times) power, was significantly smaller for Radioactivity of the hypoxia-treated rats. excised thyroid gland of hypoxia-exposed rats was less than that of controls, but differences were not significant. Thyroid to serum ratio of radioactive iodide was also less for hypoxiatreated rats but not significantly different. In contrast, the lower conversion ratio of the

hypoxia-treated rats was significantly (P < .05)different from controls. In series 3, ratio of thyroid weight to body weight, radioactivity of the excised thyroid gland, and ratio of radioactive iodide in the thyroid to that in the serum were significantly less in hypoxia-treated rats than in controls. Acinar cell height of the thyroid gland was less in hypoxia-treated rats, but the difference from control value was not significant. It is noteworthy that the ratio of thyroid weight to body weight for the rats of series 3 was approximately half that of the rats in series 1. We can advance no explanation for this difference. In general, however, the data suggest that exposure to an atmosphere containing 13% oxygen for 11 weeks reduces some aspects of thyroid function. All the changes observed in thyroid function of hypoxia-treated rats could be explained by a decrease in secretion of thyroid-stimulating hormone by the pituitary gland.

Mean weights of the organs removed at death are shown in table IV for 12 control and 11 hypoxia-treated rats (series 1 and series 3). No significant differences were observed between organ weights of control and hypoxia-treated rats. It is of interest that the significant protection against hypertension afforded by exposure to hypoxia did not manifest itself in a significant reduction in heart size. It may be that the right heart hypertrophy,

Group	Thyroid weight to body weight ratio (mg./100 gm.)	Acinar cell height (µ)	Thyroid radioactivity (% inj. dose)	I ¹³¹ Thyroid to serum ratio (c.p.m./c.p.m./ml.)	Conversion ratio
Series 1*					
Control (6)†	$8.9 \pm 0.7 \ddagger$	9.6 ± 0.5	4.7 ± 0.7	266 ± 32	74.1 ± 2.7
Hypoxia (5)	8.8 ± 0.9	8.1 ± 0.3 §	3.5 ± 0.3	188 ± 31	65.8 ± 2.3§
Series 3 ^{//}					
Control (6)	4.9 ± 0.2	9.2 ± 0.3	5.4 ± 0.5	103 ± 10	
Hypoxia (6)	4.3 ± 0.1 §	8.9 ± 0.4	3.8 ± 0.5 §	72 ± 7 §	

 TABLE III

 Effect of chronic exposure to hypoxia on thyroid activity of rats

*Killed 50 hours after injection of I¹⁸¹.

Number of rats.

‡One standard error of mean.

Significantly different from control level (P < .05).

||Killed 105 hours after injection of I181.

TABLE IV

Ratio of organ weight to body weight for certain organs of rats exposed chronically to hypoxia

Group	Mean		Orga	n weight to be	dy weight ra	tio (mg./100	gm.)	
Group	(gm.)	Heart	Kidney	Testis	Adrenal	Thymus	S. vesicle	Prostate
Control (12)*	438	322.0 ± 7.7 †	686.2 ± 18.8	795.3 ± 16.2	14.8 ± 0.9	65.8 ± 5.4	154.8 ± 6.9	138.7 ± 8.4
Hypoxia (11)	417	295.8 ± 29.4	717.4 ± 11.4	811.6 ± 21.8	15.1 ± 0.9	64.1 ± 4.0	159.5 ± 13.4	150.6 ± 14.2

*Number of rats.

tone standard error of mean.

which often accompanies exposure to hypoxia, masked the expected lower heart weight of this group. The serum sodium, potassium, and chloride concentrations measured in the plasma of the rats at death were not significantly different in the two groups. Unfortunately the rats were not killed immediately after removal from hypoxia but at either 50 or 105 hours after removal from hypoxia. This intervening period of time may have altered the pattern existing at the time of removal from hypoxia. In contrast, the serum cholesterol concentration of hypoxia-treated rats was significantly greater than that of controls, a change consistent with a possible reduction of thyroid activity of the former.

4. DISCUSSION

Rats rendered hypertensive and subjected to an atmosphere containing 13% oxygen gain significant protection against development of hypertension. This suggests that either one or a number of the physiologic changes which occur in response to hypoxia may contribute to the protection afforded by hypoxia.

The thyroid gland was chosen for study because results of earlier experiments indicated that this gland contributes, at least secondarily, to the development of hypertension in rats (4). The possibility existed that hypoxia depressed activity of the thyroid gland and therefore minimized thyroid contribution to development of hypertension. The studies of thyroid activity carried out here at least suggest that a depression of thyroid activity occurred in the rats exposed to hypoxia. The studies of Verzar et al. (16), Gordon et al. (17), and Van Middlesworth (18) support this observation. The protection against hypertension afforded by hypoxia may be due either in whole or in part to the depression of thyroid activity observed. In spite of the general agreement of the studies cited with respect to apparent reduction of activity of the thyroid glands of rats exposed to hypoxia, studies of basal metabolic rate have seldom shown a change except at very high altitudes (19, 20). The dissociation between total-body metabolic rate and handling of radioactive iodide by the thyroid gland under these conditions is perplexing and deserving of further study.

Other physiologic changes occurring in response to hypoxia may contribute to protection against hypertension (e.g., changes in acid-base balance). Although such studies were not made in the present experiments, it is possible that renal compensation of the respiratory alkalosis of the treated rats prevents the gradual accumulation of sodium and expansion of extracellular volume which may accompany development of hypertension (21). The possibility also exists that renal compensation of respiratory alkalosis is brought about by a reduced secretion of aldosterone, the adrenocortical hormone which regulates renal retention of sodium and potassium. Decreased secretion of aldosterone could contribute to the protection afforded by hypoxia against the development of hypertension since hypersecretion of aldosterone is often implicated as an etiologic factor in development of hypertension (22).

The study reported here represents preliminary observations of the effect of hypoxia on development of hypertension in rats. The various physiologic changes occurring during hypoxia need to be assessed to clarify their roles in protection against hypertension.

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