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THYROIDAL DYSFUNCTION DURING SIMULATED ALTITUDE CONDITIONS*

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Previous studies have indicated various degrees of thyroidal dysfunction in rats during exposure to high altitudes usually by simulated techniques (1-8). The present study was undertaken to determine whether acute exposure to hypoxic conditions of simulated 15,000 ft. (429 mm Hg), exerts its effect on the trapping of ^{131} iodine and/or the processes of organification in the biosynthesis of thyroxine and its release from the thyroid gland of the rat. The data obtained in the present study indicated that the conversion of radiomonoiodotyrosine (MIT-^{131}I) to radiodiiodotyrosine (DIT-^{131}I) and the synthesis of radiothyroxine (T_4-^{131}I) were significantly inhibited by hypoxia. In addition, total plasma radioactivity (^{131}I) and newly formed protein bound iodine (PB^{131}I) from hypoxic rats were significantly lower compared with control animals.

MATERIALS AND METHODS

Sprague-Dawley rats of the Charles River strain with an initial body weight of 150-200 gm were employed for all studies. Animals were fed a low iodine diet either as pelleted or powdered test form for 12 - 49 days. All animals used in a specific experiment were on the diet for the same length of time. The powdered iodine test diet contained 0.18 ug of iodine per gram of diet**(Low Iodine Test Diet, Nutritional Biochemical Corp., Cleveland, Ohio) and was used for all studies with the exception of the 23.5 hour and 28 hour studies. The pelleted form was not chemically analyzed and was used in the 23.5 hour and 28 hour studies. Water was allowed ad libitum. For each experiment, 4-8 rats were placed in individual

*The principles of laboratory animal care as established by the National Society for Medical Research were observed.
**Assayed by Graham Laboratories, Inc., Washington Street, Brookline Massachusetts.

chambers at a barometric pressure of 429 mm Hg (equivalent to 15,000 feet altitude) ventilated with a regulated air flow of 1.5 liters per minute. Equal number of rats were placed in similar chambers maintained at atmospheric pressure. After a specific interval of time (1 to 24 hours), the experimental animals were returned to atmospheric pressure. All animals then were given $Na^{131}I$ (1.1 to 2.6 uc) intraperitoneally. The experimental rats were brought back to 429 mm Hg for an additional 16 hours. All animals were sacrificed by exsanguination under light ether anesthesia. The thyroids were removed, weighed, counted in a Packard Auto-Gamma Spectrometer and hydrolyzed at pH 7.4 for 24 hours with pancreatin in 1 ml of Krebs-Ringer phosphate medium with added propylthiouracil (10^{-3}M). Twenty-five (25) microliter aliquots of digest were chromatographed in the following solvent systems: butanol-acetic acid-water (75-10-15) ascending, t-amyl alcohol saturated with 2N NH_4OH descending and t-butanol-2N NH_4OH (4-1) descending. The strips were radioautographed for identification of the ^{131}I containing spots. Each radioactive area was cut from the strip and counted in a NaI (TL) well system. A sample of serum from each animal was treated with 10 volumes of 15% cold trichloroacetic acid (TCA) to precipitate the proteins. This was then centrifuged and washed twice with 1% TCA and the precipitate assayed for radioactivity (protein bound ^{131}iodine-PB^{131}I). A sample of untreated serum was assayed to determine the total plasma ^{131}I radioactivity. Analysis of variance and/or student's t test, non-paired, were used to analyze the statistical significance of the data (9).

RESULTS

I. Thyroidal uptake of ^{131}I (Table 1): Rats exposed to simulated altitude for 1 to 24 hours prior to receiving ^{131}I and re-exposed to hypoxic conditions for an additional 16 hours showed no statistically significant alteration in uptake of ^{131}I compared with ambient pressure controls.

II. Chromatographic analysis of ^{131}I in thyroid digest (Table 2): Chromatographic results indicated that the thyroid glands from rats at altitude for 23.5 hours had a statistically significant increase in the relative formation of radiomonoiodotyrosine (48.7 $\pm$ 1.5% vs 43.0 $\pm$ 1.2%; $P<0.001$) compared with controls. A statistically significant reduction in radiothyroxine synthesis was also observed in the digest from the hypoxic animals (2.8 $\pm$ 0.52% vs 6.6 $\pm$ 0.33%; $P<0.001$) following 28 hours at altitude. After 40 hours of exposure the synthesis of radiotriiodothyronine appeared to be significantly enhanced (12.6 $\pm$ 0.9% vs 9.0 $\pm$ 1.3%; $P<0.05$) compared with the ambient pressure controls. The chromatography data did not indicate any statistically significant change in the level of gland iodide between control and experimental animals following various exposure periods.

IIa. Effect of altitude on the ratio of MIT-^{131}I/DIT-^{131}I and T3-

$^{131}I/T_4$-^{131}I (Table 3): Hypoxic animals indicated an enhanced MIT-^{131}I/DIT-^{131}I ratio following 23.5 hours of exposure (1.79 vs 1.47; $P<0.001$). The ratio of T_3-$^{131}I/T_4$-^{131}I was also increased in the experimental glands after 28 hours at altitude (3.97 vs 1.17; $P<0.001$).

III. Effect of hypoxia on plasma radioactivity (^{131}I) and protein bound iodine -PB^{131}I. Table 4: Exposure to altitude for 17 hours resulted in a significant reduction in total plasma radioactivity and protein bound radioiodine (0.83 ± 0.15 vs 2.13 ± 0.39; $P<0.01$) and 59.8 ± 7.5 vs 81.2 ± 4.7; $P<0.02$) respectively. In the present study changes in plasma radioiodine and in PB^{131}I levels in the hypoxic animals were detected prior to alterations in thyroxine biosynthesis by the thyroid gland.

DISCUSSION

The elevated thyroidal MIT/DIT ratio as seen in the control animals is probably due to iodine deficiency. Normally, one would expect the thyroidal MIT/DIT ratio in rats on an adequate iodine diet to be less than one (10). In addition, it has been reported (11, 12, 13) that iodine deficiency in vivo leads to the formation of more MIT and DIT and more T_3 relative to T_4 (11, 12). The increase in uptake of radioiodine and the enhancement in the MIT-^{131}I/DIT-^{131}I and T_3-$^{131}I/T_4$-^{131}I ratios, tables 1 and 3, as seen in both groups of animals used for the 23.5 hour, 28 hour, and 40 hour studies were probably due to the length of time on the iodine poor diet. These animals were on the diet for a longer time compared with the rats used for the 17 hour, 19 hour, and 20 hour and 21 hour studies. Accordingly, the former group of animals had an increased gland weight compared with the latter group. Assuming, therefore, in an iodine deficient rat that nearly all the radioiodine taken up by the thyroid gland is ultimately converted to hormonally active material, an increase in uptake would be reflected by an increase in the formation and release of thyroid hormones. Since within each experiment the length of dietary control was constant, comparisons in the effect of hypoxia on the thyroid gland and plasma radioactivity levels are appropriate only within each experimental period. Therefore, it is important to realize that the rats used in the present study did appear to show signs of thyroidal iodine deficiency, prior to exposure to simulated altitude. The changes reported in this study in the distribution of radioiodotyrosines and radioiodothyronines within the thryoid gland are relative changes and not absolute ones.

Since hypoxia did not result in significant changes in ^{131}I accumulation or in chromatographically identified inorganic ^{131}I but was associated with an increase in MIT-^{131}I, no depressing effect of hypoxia on the conversion of radioiodide to MIT-^{131}I was observed in the present study. However, the study did indicate an effect of hypoxia on the relative conversion of radiomonoiodocyrosine to

radiodiiodotyrosine. This observation suggested that an initial effect of hypoxia in the thyroid gland was on the biosynthesis of thyroxine and was evident in the conversion of radiomonoiodotyrosine to radiodiiodotyrosine. This step in thyroxinogenesis appeared to be more sensitive to hypoxia than the conversion of iodide to monoiodotyrosine. Nelson and Anthony (14) have reported that following 32 hours at 18,000 ft they observed an increased MIT/DIT ratio along with a decreased T_4 level in hypoxic rats.

As the period of exposure was lengthened a change in the ratio of radiotriiodothyronine to radiothyroxine was observed. The mechanism responsible for this shift in relative distribution of T_3 to T_4 might be associated with the change in MIT/DIT ratio. Slingerland et al (10) using an antithyroid drug, induced an increased MIT/DIT ratio and suggested that in presence of an increased MIT plus a reduced DIT, the synthesis of T_3 is relatively facilitated over that of T_4 and the T_3/T_4 ratio is increased. It should be mentioned, however, that the relative reduced T_4 levels observed might be partially associated with duration of fasting. Grossie and Turner (15) have reported that both the degree and duration of food restriction affects T_4 secretion in the rat. Thus, the relative alteration in the biosynthesis of thyroxine under hypoxic conditions must be carefully interpreted in light of associated changes which could occur by other means than by hypoxia alone.

Although there was no detectable change in thyroidal ^{131}I accumulation during 17 hour exposure to altitude, there was a significant decrease in plasma radioactivity and in $PB^{131}I$. Of particular interest to this observation is the published work of Keating et al (16) and Rawson (17). They showed that thyroid stimulating hormone (TSH) increased both the uptake of radioiodine in the thyroid and the release of the hormone from the gland. However, they regard the release of the hormone as the primary event and the increase of radioiodine uptake as a secondary phenomenon induced by this release. Thus, should hypoxia moderate the rate of release of TSH, the initial effect would be seen on the rate of hormonal release from thyroid gland. This would result in a reduced plasma $PB^{131}I$ level in the animals and could occur prior to any detectable alteration in thyroidal ^{131}I uptake or organification. The concentration of $PB^{131}I$ in the plasma is a complex function which depends not only on levels of TSH but also on the following factors: the proportion of dose accumulated by the gland, the rate of secretion of hormonal iodine, the amount of organic iodine in the thyroidal and extrathyroidal pools and the rates of degradation and fecal excretion of organic iodine. An increased plasma volume in the hypoxic animals would not be expected to contribute materially to the observed difference in plasma radioactivity and $PB^{131}I$ values. Thus, the mechanism responsible for the decrease in plasma radioactivity and $PB^{131}I$ following acute exposure remains to be determined.

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TABLE 1. EFFECT OF SIMULATED ALTITUDE ON THYROIDAL UPTAKE OF ^{131}I.

Time at Altitude			Thyroidal Uptake
Pre-^{131}I	Post-^{131}I		% of dose/mg thyroid
Hrs.			
1	16	C(13)	1.60 ± 0.11*
		A(13)	1.43 ± 0.18
			N.S.
3	16	C(8)	1.50 ± 0.13
		A(8)	1.20 ± 0.19
		P	N.S.
4	16	C(12)	1.06 ± 0.13
		A(12)	1.01 ± 0.26
		P	N.S.
5	16	C(16)	0.85 ± 0.11
		A(16)	1.24 ± 0.09
		P	N.S.
7.5	16	C(32)	1.78 ± 0.13
		A(32)	1.77 ± 0.11
		P	N.S.
12	16	C(16)	1.75 ± 0.19
		A(16)	1.55 ± 0.08
		P	N.S.
24	16	C(7)	1.80 ± 0.35
		A(7)	1.21 ± 0.10
		P	N.S.

C = control animals. The figure in parenthesis represents number of animals per group
A = simulated altitude (hypoxic) animals
P = probability that the mean of the altitude group is not statistically different than control group ($P > 0.05$)
* = mean ± standard error of mean

TABLE 2. EFFECT OF SIMULATED ALTITUDE ON DISTRIBUTION OF ^{131}I IN IODOTYROSINES AND IODOTHYRONINES OF HYDROLYZED THYROID GLANDS

Altitude-Hrs ^{131}I			% Total ^{131}I on Chromatogram					
Pre	Post		Butanol-Acetic Acid - H_2O*				T-Butanol - 2N NH_4OH**	
			I	MIT	DIT	Front	T_3	T_4
1	16	C(7)	16.1 ± 1.3	38.5 ± 0.46	27.3 ± 1.9	15.3 ± 1.1	13.3 ± 2.5	19.7 ± 1.9
		A(7)	18.4 ± 1.4	33.2 ± 1.0	27.9 ± 1.4	20.4 ± 0.07	20.1 ± 0.06	21.8 ± 2.0
		P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
3	16	C(8)	7.8 ± 1.1	42.0 ± 3.6	27.7 ± 2.3	21.4 ± 4.1	9.3 ± 1.7	12.3 ± 1.2
		A(8)	10.3 ± 1.3	34.3 ± 3.3	31.3 ± 1.1	24.1 ± 3.6	7.8 ± 1.8	12.2 ± 1.9
		P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
4	16	C(8)	10.9 ± 1.4	44.3 ± 5.4	23.1 ± 1.4	21.8 ± 5.7	5.9 ± 0.84	7.9 ± 1.2
		A(8)	9.5 ± 2.3	43.8 ± 4.6	20.5 ± 1.9	26.1 ± 4.6	4.8 ± 1.1	6.1 ± 1.4
		P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
5	16	C(16)	8.5 ± 1.6	44.2 ± 2.0	31.9 ± 1.9	15.3 ± 2.5	6.2 ± 1.1	14.9 ± 1.9
		A(16)	6.6 ± 0.6	46.2 ± 1.3	35.3 ± 2.0	11.8 ± 1.8	5.8 ± 1.1	10.6 ± 0.9
		P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
7.5	16	C(32)	10.6 ± 0.8	43.0 ± 1.2	30.3 ± 1.0	16.0 ± 1.2	13.2 ± 1.7	8.3 ± 0.6
		A(30)	10.1 ± 0.7	48.7 ± 1.5	28.0 ± 0.8	13.5 ± 1.0	16.8 ± 3.0	7.2 ± 0.6
		P	N.S.	< 0.001	N.S.	N.S.	N.S.	N.S.
12	16	C(16)	12.6 ± 1.1	41.6 ± 0.91	25.2 ± 1.3	20.6 ± 1.1	7.5 ± 0.66	6.6 ± 0.33
		A(16)	14.6 ± 1.1	51.1 ± 1.6	23.7 ± 1.0	10.5 ± 1.1	8.9 ± 0.84	2.8 ± 0.52
		P	N.S.	< 0.001	N.S.	< 0.001	N.S.	< 0.001
24	16	C(7)	12.8 ± 1.2	45.8 ± 2.1	25.5 ± 1.5	15.9 ± 1.51	9.0 ± 1.3	7.9 ± 1.2
		A(7)	13.7 ± 0.5	48.9 ± 2.5	22.5 ± 1.7	14.8 ± 1.46	12.6 ± 0.9	4.1 ± 0.5
		P	N.S.	N.S.	N.S.	N.S.	< 0.05	< 0.02

*I^-, MIT, DIT, Front determined in Butanol - Acetic Acid - H_2O system.
**T_3, T_4 determined in either t-Amyl-2N NH_4OH or t-Butanol-2N NH_4OH system.

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TABLE 3. EFFECT OF SIMULATED ALTITUDE ON THE RATIO OF RADIOMONOIODOTYROSINE TO RADIODIIODOTYROSINE AND RADIOTRIIODOTHYRONINE TO RADIOTHYROXINE

Time at Altitude Pre-^{131}I Hrs	Post-^{131}I	MIT-^{131}I/DIT-^{131}I	T_3-^{131}I/T_4-^{131}I
1	16	C(7) 1.41 A(7) 1.19 P N.S.	0.66 0.87 N.S.
3	16	C(8) 1.65 A(8) 1.12 P N.S.	0.78 0.67 N.S.
4	16	C(8) 1.97 A(8) 2.25 P N.S.	0.78 0.86 N.S.
5	16	C(16) 1.44 A(16) 1.34 P N.S.	0.41 0.54 N.S.
7.5	16	C(32) 1.47 A(30) 1.79 P < 0.001	1.74 2.33 N.S.
12	16	C(16) 1.76 A(16) 2.24 P < 0.05	1.17 3.97 < 0.001
24	16	C(7) 1.86 A(7) 2.25 P N.S.	1.31 3.49 = 0.01

TABLE 4. EFFECT OF HYPOXIA ON PLASMA RADIOACTIVITY (^{131}I), AND PROTEIN BOUND IODINE ($PB^{131}I$)

Time at Altitude Pre-^{131}I Hrs	Post-^{131}I Hrs		Total Plasma Radioactivity (^{131}I) % dose/10 ml plasma	% of Total Plasma Radioactivity as: $PB^{131}I$
1	16	C(12)	2.13 ± 0.39	81.2 ± 4.7
		A(12)	0.83 ± 0.15	59.8 ± 7.5
		P	< 0.01	< 0.02
4	16	C(8)	4.43 ± 0.53	93.6 ± 0.80
		A(8)	1.15 ± 0.33	78.8 ± 5.2
		P	< 0.001	< 0.02
5	16	C(16)	3.06 ± 0.38	87.1 ± 4.5
		A(16)	0.80 ± 0.11	62.4 ± 7.3
		P	< 0.001	< 0.01
7.5	16	C(31)	5.78 ± 0.71	88.8 ± 2.6
		A(31)	2.64 ± 0.26	70.4 ± 3.9
		P	< 0.001	< 0.001
12	16	C(16)	6.68 ± 0.81	92.3 ± 0.82
		A(16)	3.67 ± 0.76	74.9 ± 4.3
		P	< 0.01	< 0.001
24	16	C(7)	2.76 ± 0.60	90.6 ± 3.7
		A(7)	2.56 ± 0.74	93.6 ± 0.9
		P	N.S.	N.S.

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