STIMULATION OF PURINE SYNTHESIS BY THYROID HORMONES IN A SOLUBLE FRACTION OF RAT LIVER

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Received August 3, 1964

This communication presents the results of preliminary experiments which demonstrate that thyroid hormones stimulate the synthesis of purines.

Sprague-Dawley male rats were fed a diet containing 1% sulfaguanidine until growth arrest was established. After the 5th week of goitrogen feeding, such rats gain less than 3 gm/week and weigh 110-140 gm (Ackerman, 1963) due to an almost complete cessation of thyroid hormone synthesis.

Livers from such rats were homogenized with 2.5 volumes of Tris-phosphate buffer, pH 7.6 for 30 seconds and then centrifuged at 100,000 x g for 1 hour. Aliquots of the supernatant were incubated with ribose-5-phosphate (Sigma Chemical Co.) glycine-1-C^{14} (New England Nuclear Corp., 8 μc/umole), cofactors necessary for the synthesis of purine nucleotides, and thyroid hormones. After incubation and cooling, cold trichloracetic acid was added and the supernatant was hydrolyzed with HCl. Purines were isolated according to Abrams and Bentley (1959). The purine precipitate, dissolved in HCl and made to volume, was scanned in the ultraviolet region of the spectrum and an aliquot was then counted for radioactivity in a Packard, Tri-Carb liquid scintillation counter.

Separation of the purines was accomplished by stepwise elution of a sample on a Dowex 50-H^+ (10 x 0.07 cm^2) column with HCl. The ultraviolet absorbing fractions were pooled for identification by their absorbancy in acid and alkaline solutions and by paper chromatography. Seven fractions were repeatedly obtained. Adenine, guanine and hypoxanthine were considered pure by the above criteria. Uric acid appeared in a mixture of two unidentified

substances. Also, three additional fractions were unidentified, but these exhibited no characteristic absorption spectra and incorporated little radioactivity. These were ignored.

As shown in Table 1, the addition of L-thyroxine (L-T_4) to the system described, resulted in an increase in absorbancy at 260 mμ and an increase in incorporated radioactivity. The omission of $NaHCO_3$ or NAD and GTP, or the addition of 6-mercaptopurine (6MP), did not alter the response to thyroxine.

Table 1. Incorporation of Glycine-C-14 into Purines of the Soluble Fraction of Rat Liver

	Absorbancy at 260 mu		Radioactivity of Purines cpm/total purines	
Experiment	$-T_4$	$+T_4$	$-T_4$	$+T_4$
1	0.502	0.882	4025	6149
2	0.570	0.973	3768	6422
3	0.625	1.034	3075	5341
4. - $NaHCO_3$	0.532	0.960	2065	2545
5. - NAD , - GTP	0.543	0.865	1983	5346
5. + 6MP*	0.906	1.302	7516	8754

The system contained 1.0 ml of the 100,000 x g supernatant fraction of rat liver, 1 mmole of Tris-phosphate, pH 7.6, glycine-1-C-14 (400,000 cpm) and in μmoles: KCl, 60; $MgCl_2$, 15; $NaHCO_3$, 60; succinate, 15; glutamine, 30; ribose-5-phosphate, 60; aspartate, 60; mercaptoethanol, 60; ATP, 3; GTP, 3; NAD , 3; tetrahydrofolic acid, 10; and T_4 when added, 75, in a total volume of 3.0 ml. Incubated in air for one hour at 37°. Purines were isolated after acid hydrolysis by precipitation with silver from alkaline solutions.
* Experiment 5 but with 2.6 umoles 6-mercaptopurine added.

Using the system described for experiments 1 to 3 of Table 1, the concentration of T_4 was varied as follows: 0, 2.5×10^{-7}, 2.5×10^{-6}, 2.5×10^{-5} and 2.5×10^{-4} molar. The radioactivities of the isolated purines were: 3268, 3403, 3743, 4758 and 4110 cpm, respectively. Using 3,5,3'-triiodo-L-thyronine (T_3) instead of T_4 in the following concentrations: 0, 2.5×10^{-8}, 2.5×10^{-7},

2.5×10^{-6}, 2.5×10^{-5} molar, the radioactivities of the purine fractions were: 1983, 17,503, 15,742, 7592 and 5317 cpm, respectively. D-thyroxine in concentrations of 0 and 2.5×10^{-5} molar resulted in 4025 and 4859 cpm, respectively, in the purine fractions.

These results show that both T_4 and T_3 stimulate the incorporation of glycine-1-C^{14} into purines. The greater efficacy of T_3 and the lesser effect of D-T_4 with respect to L-T_4, are in line with the physiological effects of these compounds as summarized by Pitt-Rivers and Tata (1959). We believe the results to be significant because the soluble enzyme systems were obtained from growth-arrested, thyroid hormone deficient rats. Heretofore, the addition of T_4 to in vitro systems obtained from thyroid hormone deficient rats generally produced little or no response (Sokoloff and Kaufman, 1961; Stein and Gross, 1962).

Table 2. Concentration and Specific Activities of the Purines Isolated from the Soluble Fraction of Rat Liver

	Experiment 1		Experiment 5			
	$-T_4$	$+T_4$	$-T_4$	$+T_4$	+6MP* $-T_4$	+6MP* $+T_4$
Adenine						
µmoles	1.10	1.37	0.575	0.795	0.920	1.574
cpm/µmole	208	263	305	363	302	448
Guanine						
µmoles	0.843	0.665	0.313	---	0.101	---
cpm/µmole	360	312	1210	---	1088	---
Hypoxanthine						
µmoles	1.51	0.628	2.31	0.843	1.73	0.723
cpm/µmole	3711	4824	148	447	1386	1982
"Uric Acid"						
A at 285 mµ	0.275	0.595	0.145	0.375	0.445	0.258
cpm/A at 285 mµ	1918	621	3326	710	5626	3129

The purine fractions obtained from experiments 1 and 5 as described in Table 1, were separated on a Dowex 50-H^+ column by stepwise elution with 50 ml each of 0.1 N HCl, 0.2 N HCl, 0.3 N HCl and 1.0 N HCl. "Uric acid" was a mixture of 3 ultraviolet absorbing substances. The absorbancy (A) of the entire mixture and its radioactivity were determined.
* 6-mercaptopurine.

Separation of the purines obtained from experiment 1 of Table 1 revealed that the addition of T_4 had resulted in an increase in the total quantity of isolated adenine and an increase in its specific activity (Table 2). However, guanine had decreased, as did its specific activity. Hypoxanthine also decreased, but its specific activity had increased. The uric acid fraction had increased, but its specific activity had decreased.

The omission of GTP (necessary for AMP synthesis) and of NAD (necessary for GMP synthesis) would be expected to increase the concentration of hypoxanthine (from IMP) and its specific activity. This did not occur. The same pattern of results were obtained in experiment 5 as was observed in experiment 1. When T_4 was added to the system, guanine decreased to the point where it was undectable, and again hypoxanthine decreased but with an increase in its specific activity. The same pattern of results were again observed when 6MP was included in the media with the exception that uric acid had decreased. 6MP was expected to inhibit the synthesis of IMP from its precursors and of AMP from IMP.

The results of these experiments suggest to us that the thyroid hormones were stimulating the synthesis of AMP from IMP at the expense of GMP. A stimulation of this reaction would result in a decrease in the concentration of IMP and in a decrease of GMP because of a decreased rate of its synthesis and its continued conversion to uric acid and other products. The increase in the specific activity of hypoxanthine would be a result of the continued synthesis of IMP from glycine-C^{14}.

This work was supported by a grant from the National Institutes of Health (AM 08175).

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