THYROTROPIN STIMULATION OF PYRIMIDINE NUCLEOTIDE SYNTHESIS IN BOVINE THYROID

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Hall and Tubmen (1963, 1965, 1965a) demonstrated that thyrotropin (TSH) increases the uptake of ^{14}C-formate, ^{14}C-adenine and ^{14}C-glycine into acid-soluble and RNA purines in bovine thyroid slices and interpreted their data as indicating a stimulation of purine nucleotide synthesis resulting in increased RNA formation. The TSH effect was attributed to an increase in ribose which occurs as a result of TSH stimulation of the hexose monophosphate shunt. According to this hypothesis, the stimulation of nucleic acid synthesis is a consequence of the TSH effect on glucose oxidation and not due to a primary effect on nucleic acid synthesis. However, Lecocq and Dumont (1967, 1967a) as well as Shimada and Yasumasu (1966) have shown that ^{3}H-uridine incorporation into RNA is also stimulated by TSH in porcine and sheep thyroid slices indicating that the incorporation of precursors not requiring ribose may also be affected by this hormone. In addition, Begg and Munro (1965, 1965a) and Shimada and Yasumasu (1966) have reported that TSH stimulated the incorporation of ^{14}C-UTP into RNA in isolated thyroid nuclei and may possibly affect RNA polymerase or the "primer" activity of thyroid chromatin for RNA synthesis.

Hall's conclusion that TSH stimulated purine synthesis was based on increases in the specific activities of acid-soluble adenine and hypoxanthine when tissue slices were incubated in the presence of ^{14}C-

formate. However, phenomena other than an increase in synthesis, such as a decrease in the tissue pool of purines, could produce an increase in specific activity. In view of the TSH increase in tissue utilization of purines (Hall, 1963) and of nucleosides and nucleotides for RNA formation, a decrease in the tissue pool of purines might very well occur.

The validity of Hall's conclusion has been investigated in a system which makes it possible to measure the total amount of pyrimidine nucleotide formed from orotic acid-carboxyl-^{14}C by determining the $^{14}CO_2$ produced upon decarboxylation of OMP to form UMP. The results obtained demonstrate that TSH stimulates pyrimidine nucleotide synthesis confirming that this hormone affects nucleotide formation in addition to the reported effects on polymerization of nucleotides to form RNA.

MATERIALS AND METHODS

Bovine thyroids were obtained from the abattoir, freed of fat and sliced with a Stadie-Riggs slicer. The slices were placed on buffer-moistened filter paper in covered, iced petri dishes prior to the final removal of extraneous fat and weighing. Thyroid slices (100 mg ± 3.0 mg) were incubated in triplicate in 3.0 ml of Krebs-Ringer bicarbonate medium containing 8 μmoles of orotic acid and, when present, 1.0 unit of TSH, 4.0 μmoles of glucose and various concentrations of azauridine. Incubation was carried out in stoppered flasks with a removable center well at 37°C in 95% oxygen - 5% CO_2 at a shaking rate of 120/min. Hyamine (1.0 ml) was injected into the center well and the reaction terminated by the injection of 0.3 ml 4 N perchloric acid. The vessels were shaken for an additional 60 minutes for $^{14}CO_2$ collection. The hyamine was then quantitatively transferred to scintillation vials and counted in a Packard Tricarb scintillation spectrometer.

Orotic acid carboxyl-^{14}C, 3.67 μc/μmole and glucose-1-^{14}C, 3.95 μc/μmoles were obtained from New England Nuclear Corporation, TSH (1 unit/mg

dry wt.) from Princeton Laboratory Products and azauridine from Nutritional Biochemical Corporation.

RESULTS AND DISCUSSION

The utilization of orotic acid for nucleotide formation involves the enzymatic combination of this pyrimidine with 5-phosphoribosylpyrophosphate (PRPP) to form OMP which is decarboxylated forming UMP (Lieberman et al., 1955). The UMP may then be converted to other pyrimidine nucleotides and RNA. If orotic acid-carboxyl-^{14}C is used, this two-step conversion to UMP results in the stoichiometric loss of the carboxyl carbon as $^{14}CO_2$ and the $^{14}CO_2$ formed is indicative of the total amount of pyrimidine nucleotides synthesized from orotic acid.

In order to establish that the loss of $^{14}CO_2$ from orotic acid-carboxyl-^{14}C in bovine thyroid slices was a result of the decarboxylation of OMP and not due to degradation to $^{14}CO_2$ by other pathways, the effects of azauridine were investigated. Azauridine is converted to azauridine-5'-monophosphate by uridine kinase, an enzyme widely distributed in thyroid and other tissues. The azauridine-5'-monophosphate formed is a specific inhibitor of OMP decarboxylase (Skoda, 1963) and consequently inhibits the conversion of orotic acid to all pyrimidine nucleotides used for nucleic acid synthesis.

Bovine thyroid slices were preincubated for 1 hour with various concentrations of azauridine (to allow time for azauridine-5'-monophosphate accumulation) or with TSH prior to the addition of orotic acid-carboxyl-^{14}C or glucose-1-$^{14}CO_2$ and subsequent incubation for 1 additional hour. The results are presented in Table 1. An inhibition of $^{14}CO_2$ formation from orotic acid-carboxyl-^{14}C of 85.1% was produced by 1 μmole of azauridine and increased to 98.4%, essentially complete inhibition, when the azauridine concentration was raised to 20 μmoles. However, 20 μmoles of azauridine had no effect on the oxidation of glucose-1-^{14}C, a parameter

TABLE 1

THE EFFECTS OF AZAURIDINE ON $^{14}CO_2$ PRODUCTION FROM OROTIC ACID-CARBOXYL-^{14}C IN BOVINE THYROID SLICES

Bovine thyroid slices were preincubated for 1 hour in 3.0 ml Krebs-Ringer bicarbonate medium containing 4 μmoles of glucose, 8 μmoles orotic acid, various concentrations of azauridine and, when present, 1 unit of TSH. Carboxyl-^{14}C orotic acid (2 μc, 0.54 μmoles) or glucose-1-^{14}C (0.5 μc, 0.13 μmole) was then added and incubation continued for 1 hour.

Azauridine added μmoles/3.0 ml	DPM $^{14}CO_2$ Recovered		
	Orotic-^{14}COOH		Glucose-1-^{14}C
	Control	TSH	
I. None	5,878		6,113
1	873		
2	735		
5	538	533	5,453
II. None	5,338	6,994	6,839
5	318		
10	179		
20	85		7,206

sensitive to alterations in the metabolic integrity of thyroid slices.

The inhibitory effect of azauridine, or more specifically azauridine-5'-monophosphate, is on OMP decarboxylase. Azauridine also competes with uridine for uridine kinase but this enzyme is not directly involved in orotic acid metabolism. Other sites of inhibition in animal tissues have not been reported. In addition, since azauridine doesn't appear to alter vital metabolic processes in thyroid slices such as glucose metabolism, it is highly unlikely that the degradation of orotic acid-carboxyl-^{14}C to $^{14}CO_2$ via pathways other than those involving OMP decarboxylation would be seriously affected by azauridine. Consequently, the $^{14}CO_2$ production inhibited by azauridine (98.4%) must represent the $^{14}CO_2$ produced by decarboxylation of OMP to form nucleotides and the $^{14}CO_2$ not inhibited by azauridine (1.6%) would represent the maximum $^{14}CO_2$ production occurring by other pathways. The 98.4% inhibition with azauridine, therefore, demonstrates that $^{14}CO_2$ production from orotic acid-carboxyl-^{14}C in bovine thyroid slices is due almost exclusively to the formation of pyrimidine

nucleotides. Supporting evidence for this observation was obtained when the amount of $^{14}CO_2$ produced from orotic acid-carboxyl-^{14}C was compared with the incorporation of radioactivity from orotic acid-6-^{14}C into uracil, uridine, uridine nucleotides and RNA. The conversion of orotic acid-6-^{14}C to these compounds in the absence of TSH was 101.3% of the $^{14}CO_2$ produced from orotic acid-carboxyl-^{14}C and 90.8% in the presence of TSH.

A TSH stimulation of the conversion of orotic acid to pyrimidine nucleotides in thyroid tissue is demonstrated by the results presented in Tables 1 and 2. In Table 1, the addition of TSH produced a 30% stimulation

TABLE 2

TSH STIMULATION OF OROTIC ACID-CARBOXYL-^{14}C
CONVERSION TO PYRIMIDINE NUCLEOTIDES

Bovine thyroid slices were incubated 2 hours in 3.0 ml Krebs-Ringer bicarbonate medium containing 8 μmoles of orotic acid-carboxyl-^{14}C (2 μc) and, when present, 4 μmoles of glucose and 1 unit of TSH.

Additions	Pyrimidine nucleotides formed		
	Control	TSH	% Change
	(Mμmoles)	(Mμmoles)	
None	21.9	28.3	29.2
Glucose	29.5	40.7	38.0

of pyrimidine nucleotide synthesis in the absence of azauridine but had no effect in the presence of 5 μmoles of azauridine. As shown in Table 2, the TSH stimulation occurred both in the presence and in the absence of glucose. The addition of glucose to the medium increased pyrimidine nucleotide synthesis over controls without carbohydrate; however, TSH produced an additional stimulation (38%) which was consistently greater than that produced in the absence of glucose (29%). This hormone consistently stimulated the conversion of orotic acid to pyrimidine nucleotides; however, the magnitude of the response varied with the thyroid gland.

The results presented in this communication demonstrate that TSH stimulates total pyrimidine nucleotide synthesis in bovine thyroid slices.

These findings along with the reports by Hall and Tubmen (1963, 1965, 1965a) strongly indicate that one site at which TSH may affect nucleic acid synthesis is the formation of nucleotides. The demonstration of a TSH enhancement of pyrimidine nucleotide synthesis does not indicate an effect on RNA; however, increased formation of RNA would be an expected consequence of an increase in the rate of synthesis of nucleotides.

Although the results presented demonstrate a TSH stimulation of nucleotide formation, the evidence presented by Begg and Munro (1965, 1965a), Shimada and Yasumasu (1966) and Lecocq and Dumont (1967, 1967a) strongly indicates another site, the polymerization of nucleotides, at which TSH can affect the synthesis of nucleic acids. Consequently, TSH appears to have a dual role on nucleic acid formation in thyroid tissue.

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