

Oestrogen Induction of Riboflavin-Binding Protein in Immature Chicks

MODULATION BY THYROID STATUS

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In hyperthyroid chicks, oestrogen-induced plasma accumulation of riboflavin-binding protein was diminished, whereas the reverse situation prevailed in hypothyroid birds. Under hyperthyroid conditions, higher concentrations of oestrogen were required to elicit a response comparable with that obtained in normal birds treated with lower concentrations of the hormone. Elevated hepatic cytochrome *P*-450 concentrations and decreased half-life of the induced protein in hyperthyroid animals suggest that higher catabolic rates of the inducer and induced protein are contributory factors to the diminished response.

The reciprocal interaction between oestrogen and the thyroid hormones at the level of metabolism and/or growth and function of target tissue is well documented. This is exemplified by (a) the counteraction of oestrogen-induced adenohypophysial and thyroid hypertrophy by thyroxine (Schriber, 1971; Yamada *et al.*, 1966), (b) the enhanced peripheral metabolism of the thyroid hormones after the administration of steroid hormones (Galton, 1971) and (c) altered uterine sensitivity to oestrogen in hyperthyroid and hypothyroid animals (Ruh *et al.*, 1970*a,b*). The findings that oestrogen induced elevated concentrations of serum alkaline phosphatase and Ca^{2+} in laying hens (Snapir & Perek, 1970) and that biotin, riboflavin and vitamin A concentrations (and hepatic hypertrophy) in immature female chicks (Common *et al.*, 1949; Mooyoung *et al.*, 1960) are severely curtailed under hyperthyroid conditions suggest that the thyroid status of an animal can influence the steroid-hormone-induced specific biochemical responses. However, the mechanisms involved have remained largely unexplored.

The chicken liver, besides being a target tissue for oestrogen in terms of induction of specific yolk proteins (Gruber *et al.*, 1976), is also a major catabolic site for the steroid, and thus represents an attractive model system for exploration of the biochemical basis of oestrogen-thyroid-hormone interaction. Riboflavin-binding protein is a yolk protein that is specifically induced after oestrogen treatment (U. S. Murthy & P. R. Adiga, unpublished work). The present communication deals with the modulation of induction of this specific protein under hyperthyroid and hypothyroid conditions and the underlying mechanisms.

Materials and Methods

Oestradiol-17 β , L-thyroxine, propylthiouracil and sodium cholate were products of Sigma Chemical Co., St. Louis, MO, U.S.A. Na ¹²⁵I for protein iodination was procured from The Radiochemical Centre, Amersham, Bucks., U.K. The sources of other chemicals have been listed elsewhere (Murthy & Adiga, 1977).

Hypothyroid and hyperthyroid conditions and induction of riboflavin-binding protein

White Leghorn male chicks (2 months old; 0.4-0.5 kg body wt.) obtained from the Central Poultry Farm, Hesserghatta, Bangalore, India, were maintained in individual cages and fed on a commercial diet and water *ad libitum*. They received six daily intraperitoneal injections of either L-thyroxine (100 μ g/kg) in saline (hyperthyroid) or propylthiouracil (100 mg/kg) in 0.5% (w/v) gelatin in saline (hypothyroid). The control birds received saline (0.9% NaCl) only. After 6 days, a single dose of oestradiol-17 β /propane-1,2-diol was administered intramuscularly to individual birds at the indicated doses (see the legends to Tables 1 and 2). Blood (0.5 ml) was withdrawn at specified times by cardiac puncture, plasma prepared and the riboflavin-binding-protein content was measured by the sensitive radioimmunoassay procedure (Murthy & Adiga, 1977).

Half-life of riboflavin-binding protein

Highly purified egg-white riboflavin-binding protein (Murthy & Adiga, 1977) was radioiodinated with Na ¹²⁵I by the method of Greenwood *et al.* (1963), purified by gel filtration on Sephadex G-25, and

diluted with unlabelled riboflavin-binding protein to a final concentration of 1 mg/ml in saline. It was then administered by the intracardiac route to the control and the hyperthyroid birds. Blood samples were withdrawn at 1 h intervals, and plasma was prepared and its radioactivity counted in an Autogamma counter (Packard Autogamma Counter, model 2002). Samples (0.2 ml) of the plasma were treated with 1:50-diluted specific rabbit antiserum to riboflavin-binding protein (Murthy *et al.*, 1976) at 37°C for 6 h, and 0.2 ml of double antibody (goat antiserum to rabbit immunoglobulin) was added to each to precipitate ¹²⁵I-labelled riboflavin-binding protein-rabbit antibody complex (Murthy & Adiga, 1977). The radioactivity in the precipitate was counted and was invariably found to represent 80% of total blood radioactivity. This procedure established that a major and constant proportion of blood radioactivity represented that due to circulating ¹²⁵I-labelled riboflavin-binding protein. The half-life of the protein was computed from the rate of disappearance of the labelled protein from the circulation (McFarlane, 1964).

Determination of hepatic cytochrome P-450

The low content of cytochrome P-450 in this system (Kulkarni *et al.*, 1976) necessitated the solubilization of the microsomal fraction (Lu & Levin, 1972). A 5–10-fold higher concentration of the protein could thus be used for the assay. A 5% (w/v) homogenate of liver (3 g) in ice-cold 1.15% (w/v) KCl was spun at 20000g for 30 min at 4°C and the microsomal fraction was sedimented from this postmitochondrial supernatant in a Beckman/Spinco model L-3 ultracentrifuge (140000g for 60 min). The microsomal pellet was suspended in 3 ml of 0.1 M-potassium phosphate buffer, pH 7.6, containing 20% (v/v) glycerol, 1 mM-EDTA and 1 mM-dithiothreitol, then sonicated twice (6 mA for 30 s; Branson Sonifier model S75), and the suspension was clarified by adding sodium cholate to the final concentration of 1% (w/v). The cytochrome P-450 was assayed as detailed by Omura & Sato (1964). Protein concentration in these preparations was measured by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin as the standard.

Results and Discussion

The kinetics of elaboration of riboflavin-binding protein in male chicks as reflected by the plasma concentration of the protein in response to a single injection of oestradiol-17 β show that, after an initial 4 h lag phase, the concentration of the protein increases severalfold by 24 h, reaching peak values around 48 h, declining in a symmetrical fashion thereafter (U.S. Murthy & P. R. Adiga, unpublished work). Further, this pattern was not qualitatively

influenced by the thyroid status of the birds. However, the data in Table 1 clearly show that quantitatively both hypothyroid and hyperthyroid conditions profoundly influence the extent of accumulation of riboflavin-binding protein in the blood, and this phenomenon is evident during both ascending (24 h) and descending (72 h) phases of the induction process. Whereas chronic thyroxine treatment drastically diminished the accumulation of riboflavin-binding protein to approx. 40% of that in chicks treated with oestrogen alone, the reverse situation prevailed when propylthiouracil interfered with endogenous thyroid-hormone production. Since all the circulating riboflavin in oestrogenized chicks is bound to the riboflavin-binding protein (U. S. Murthy & P. R. Adiga, unpublished work), the earlier findings (Common *et al.*, 1949) that oestrogen-enhanced plasma riboflavin content is curtailed by thyroxine treatment can be explained as being primarily due to decreased content of the binding protein, rather than to exclusive interference with metabolism of the vitamin as such.

Among the possible underlying mechanisms, it was considered that the diminished response to oestrogen under hyperthyroid conditions could be due to an accelerated metabolic clearance rate of the steroid with a resultant decrease in effective concentration of the inducer at the site of its action, namely the liver. The validity of this premise is supported by the finding that increasing the dose of oestradiol to 20 mg/kg body wt. in hyperthyroid birds elicited a response comparable with that obtained with 10 mg of oestrogen/kg body wt. in control chicks (Table 1).

Table 1. Influence of hypothyroidism and hyperthyroidism on oestrogen-induced plasma concentrations of riboflavin-binding protein in male chicks

Experimental details are given in the text. Preinjection control values are means of those obtained with nine chicks. Chronic treatment with thyroxine or propylthiouracil did not alter the baseline values. Experimental values are means \pm S.D. for four chicks.

Treatment (mg/kg body wt.)	Plasma concentration of riboflavin-binding protein (ng/ml)	
	At 24h	At 72h
None (control)		21
Oestrogen (10)	920 \pm 129	650 \pm 10
Propylthiouracil (100) + oestrogen (10)	1466 \pm 139*	925 \pm 75*
Thyroxine (0.1) + oestrogen (10)	316 \pm 47*	186 \pm 20*
Thyroxine (0.1) + oestrogen (20)	946 \pm 158	690 \pm 75

* Values significantly different from those obtained with oestrogen treatment alone at $P < 0.01$.

Pertinent in this connexion is the observation by Vonhorn (1933) that in hyperthyroid ovariectomized rats higher amounts of oestrogen were needed to produce a positive oestrus vaginal smear. Also consistent with our finding are the data of Ruh *et al.* (1970*a,b*), showing that the uterine uptake and retention of [^3H]oestradiol as well as weight response were higher in hypothyroid and diminished in hyperthyroid rats.

A higher rate of oestradiol catabolism, especially to its hydroxylated products, in hyperthyroid humans has been well established (Gallagher *et al.*, 1966). Furthermore steroid hydroxylation proceeds preferentially through the mixed-function oxidase system in which cytochrome *P*-450 occupies a central position (Kuntzman *et al.*, 1965), and therefore microsomal cytochrome *P*-450 concentrations should represent a sensitive index of the catabolic rate of oestradiol (Kappas & Song, 1969). The data in Table 2 clearly show that oestradiol administration leads to marked increase in the hepatic cytochrome *P*-450 content measured at 24 h. This is in agreement with the extensive proliferation of smooth (as well as rough) endoplasmic reticulum (the cellular locus of cytochrome *P*-450) that has been noticed in chickens treated with the steroid hormone (Schjeide *et al.*, 1974). Of interest in this context is the observation (Levin *et al.*, 1967) that chronic phenobarbitone treatment of rats, resulting in enhanced hepatic cytochrome *P*-450 concentrations, led to a markedly diminished response to oestrogen in terms of the production of uterine hypertrophy. More specifically, chronic thyroxine treatment augmented these concentrations further, in good agreement with the

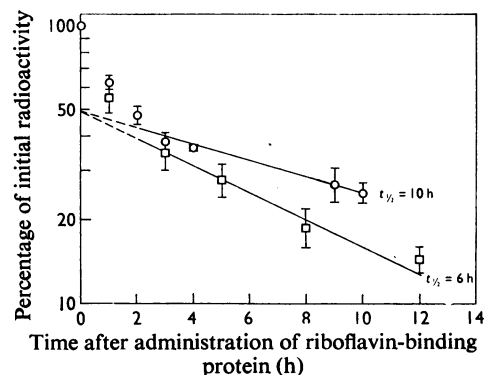


Fig. 1. Time course of disappearance of ^{125}I -labelled riboflavin-binding protein from the plasma of untreated and thyroxine-treated male chicks

Freshly ^{125}I -labelled riboflavin-binding protein (1 mg; 4×10^6 c.p.m./chick) was injected into 2-month-old untreated birds (○) or thyroxine-treated birds (□) by the cardiac route. Oestrogen administration by itself had no influence on the half-life of exogenously administered riboflavin-binding protein in control and thyroxine-treated birds. Radioactivity in the plasma was measured at 1 h intervals. Each point represents the means \pm s.d. of three to six chicks.

accelerated catabolism of oestradiol in hyperthyroidism suggested above. Thyroxine treatment in itself increased the cytochrome *P*-450 concentration significantly, and this may be related to increased utilization of endogenous substrates by the liver of the hyperthyroid birds.

Since the extent of accumulation of riboflavin-binding protein in the blood is determined by the relative rates of synthesis and degradation, it was considered that an additional contributory factor for decreased concentration of the protein in hyperthyroidism might be related to enhanced turnover rate of the induced protein. This was sought to be established by comparing the half-life of the exogenously administered ^{125}I -labelled protein in control and thyroxine-treated birds. Fig. 1 shows that in the normal or oestrogen-treated birds the $t_{1/2}$ of riboflavin-binding protein is about 10 h, whereas it is only 6 h in hyperthyroid chicks. Although perfusion studies with isolated rat liver have shown (Griffin & Miller, 1973) that the synthetic rates of different normal plasma proteins are differentially affected by thyroxine, to our knowledge the present data represent the first demonstration of the thyroid hormone drastically shortening the turnover rate of an oestrogen-induced specific protein.

From the foregoing, it is clear that enhanced catabolism of both the inducer and the induced protein are contributory mechanisms underlying the modu-

Table 2. Influence of oestrogen and thyroxine on hepatic cytochrome *P*-450 content in male chicks

Experimental details are given in the text. In the combined treatment, thyroxine was injected daily for 6 days before oestrogen; then 24 h after a single injection of oestrogen the content of cytochrome *P*-450 was determined. Values are means \pm s.d. for three chicks.

Treatment (mg/kg body wt.)	Cytochrome <i>P</i> -450 content (nmol/mg of microsomal protein)
None (control)	0.179 \pm 0.023
Oestrogen (10)	0.247 \pm 0.042*
Thyroxine (0.1)	0.297 \pm 0.061†
Thyroxine (0.1) + oestrogen (10)	0.455 \pm 0.03‡

* Value significantly different from the control at $P < 0.05$.

† Value significantly different from the control at $P < 0.02$.

‡ Value significantly different from the control at $P < 0.001$ and from that obtained with thyroxine alone or oestrogen alone at $P < 0.01$.

lation of oestrogenic response by the thyroid hormones. Yet another additional factor envisaged is the thyroid-hormone-influenced alteration in the sensitivity of the target tissue to oestrogen (Meites & Chandrashekar, 1949; Ruh *et al.*, 1970a), and this awaits further study.

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