《Research Note》

Relationships between Plasma Levels of Thyroxine and Its Binding Proteins in the Chick Embryo

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Plasma levels of thyroxine (T4), total proteins, albumin and transthyretin (T4-binding prealbumin) were determined in chick embryos at 19 days of incubation. There were significant sex differences ($P < 0.05$) in plasma levels of T4 and total proteins. A highly significant positive correlation between plasma levels of T4 and albumin ($r = 0.55$, $P < 0.001$, $n = 42$) was found; this suggests that plasma albumin level may be a useful parameter to evaluate thyroid function in the chick embryo. Moreover it was confirmed that both albumin and transthyretin were major T4-binding proteins in chick embryos as well as in growing chickens.

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Key words: thyroxine, albumin, thyroxine-binding proteins, chick embryo

Introduction

In birds, plasma thyroid hormones are bound principally to albumin and transthyretin (thyroxine-binding prealbumin) and, to a minor extent, to lipoproteins and globulins (DAVISON *et al.*, 1978; MITCHELL and STILES, 1985; COOKSON *et al.*, 1988). The association of thyroid hormones to their binding proteins may retard clearance rates of circulating hormones and support continuous synthesis and release of the hormones by decreasing plasma concentrations of free hormone that is regarded as important in the negative feedback mechanism (LARSEN, 1982). However, we lack definite information on the binding proteins of thyroid hormones in the chick embryo. Moreover, we have only scanty information about relationships between the levels of circulating thyroid hormones and their binding proteins in chickens.

Experiments reported in this communication show the existence of thyroxine (T4)-binding proteins and a positive correlation between plasma T4 and albumin levels in the chick embryo.

Materials and Methods

Animals

Fertilized eggs (Babcock B-300) were incubated in a force-draft laboratory incubator at 37.8°C with a constant relative humidity of 60% and turned once every 4 h. After incubation for 19 days, blood samples were obtained by heart puncture into

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heparinized tubes. Plasma was separated by centrifugation ($2,000\times g$, 20 min) and stored at -20°C until assayed.

Determination of plasma albumin and transthyretin concentrations

Serum albumin and transthyretin were purified from broiler sera obtained from a local processing company as described by SCHWERT (1957) and ABE *et al.* (1975), respectively. The purified proteins, both of which gave a single band on disc polyacrylamide gel electrophoresis (PAGE) (DAVIS, 1964), were used as antigens to raise antisera in rabbits in the usual manner. The antisera raised were specific to the antigens and showed no crossreactivity with the other plasma proteins and ovoalbumin in the immunoelectrophoresis (GRABAR and WILLIAMS, 1955). Using these antisera, plasma albumin and transthyretin concentrations were determined by a single-radial immunodiffusion method (MANCINI *et al.*, 1965) as follows. Plasma ($2.5\mu\text{l}$) or standard solutions (albumin, 0–20 mg/ml; transthyretin, 0–2 mg/ml) ($2.5\mu\text{l}$ each) were dispensed into holes (2 mm in diameter) on 1% (w/v) agar plate containing 1% (v/v) antiserum. The plates were incubated for 24 h at 30°C under humid condition. Diameters of the resultant precipitate rings were measured and then protein concentrations were determined from the calibration lines made with the standard solutions of albumin and transthyretin. The intraassay variations (c.v.) of albumin and transthyretin were 4.3% ($n=5$) and 5.9% ($n=5$), respectively. The assays were carried out in duplicate at one time to expel interassay variations. The calibration lines for albumin and transthyretin were $Y=0.4762+0.0968 X$ ($r=0.97$, $P<0.001$) and $Y=0.2104+0.0052 X$ ($r=0.99$, $P<0.001$) respectively, where Y is protein concentration (mg/ml) and X is (diameter of the rings in cm)².

Concentrations of total plasma proteins were measured by the method of LOWRY *et al.* (1951). Plasma T4 levels were determined by a radioimmunoassay described previously (HOSHINO *et al.*, 1993).

Detection of plasma T4-binding proteins

Plasma from 6 embryos (3 males and 3 females) was pooled. Plasma from 6-week-old chickens (2 males) was also pooled. The pooled plasma ($50\mu\text{l}$) of each group was incubated with 1 ng (200,000 cpm, dissolved in $10\mu\text{l}$ of ethanol: water = 3:1 solution) of ^{125}I -T4 (Amersham) in a plastic tube for 4 h at room temperature, and then 1 ml of 1% (w/v) sucrose solution containing 5% (w/v) activated charcoal (Norit A) was added to the tube to remove free ^{125}I -T4. Fifty μl of this mixture was applied to the disc-PAGE of DAVIS (1964) and the electrophoresis was run vertically with a constant current of 2 mA/gel. After completion of electrophoresis, gels were cut into 1.5 mm segments and their radioactivities were measured with an automatic gamma counter (ARC-600, Aloka). The pooled plasma of embryo ($5\mu\text{l}$) and of chick ($2\mu\text{l}$) were run at the same time and stained with Coomassie brilliant blue G-250 to detect proteins.

Statistics

Statistical analyses of data were made using Student's *t*-test or Cochran-Cox test.

Results

Plasma levels of T4 and blood proteins in the embryos at 19 days of incubation are

Table 1. Plasma levels of thyroxine, total proteins, albumin and transthyretin (thyroxine-binding prealbumin) in chick embryos at 19 days of incubation (mean $\pm$ SE)

	Thyroxine (ng/ml)	Total proteins (mg/ml)	Albumin (mg/ml)	Transthyretin (μ g/ml)
Female (n=18)	14.12 $\pm$ 1.22	23.45 $\pm$ 1.69	6.93 $\pm$ 0.60	72.30 $\pm$ 4.53
Male (n=24)	10.57 $\pm$ 0.96*	17.83 $\pm$ 1.74*	6.12 $\pm$ 0.33	72.82 $\pm$ 5.08

* $P < 0.05$ vs female

shown in Table 1. Both plasma T4 and total protein levels were significantly higher in female embryos than in male embryos. However, there were no significant sex differences in plasma levels of albumin and transthyretin. When data were correlated by linear regression, a significant positive correlation was found between plasma levels of T4 and albumin ($r=0.55$ for pooled data, $P < 0.001$, $n=42$; $r=0.58$ for females, $P < 0.05$, $n=18$; $r=0.46$ for males, $P < 0.05$, $n=24$). However, neither total protein nor transthyretin levels were significantly correlated with plasma T4 levels.

Patterns of ^{125}I -T4-binding to plasma proteins, together with electrophoretic profiles of proteins, are shown in Figs. 1 and 2. The radioactivities were mainly detected in albumin and transthyretin, and to some extent in the other protein fractions. The T4-binding pattern of 6-week-old chicken plasma was similar to that of embryos, though the amounts of binding to proteins, especially to albumin, tended to decrease in the growing chickens.

Discussion

The present study clearly showed a significant positive correlation between plasma levels of T4 and albumin, which appears to be a major T4-binding protein in chick embryos as well as in chickens after hatch, suggesting possible involvement of albumin in the thyroïdal function of chick embryos. For instance, it is possible that the thyrotropic activity of estrediol (E_2) observed in the previous experiments (HOSHINO *et al.*, 1987, 1990; HUYBRECHTS *et al.*, 1990) could have been sustained by an increase in albumin concentration. Estrogens are known to increase the plasma vitellogenin concentrations in cockerels (CLEGG *et al.*, 1951) and quails (GIBBINS and ROBINSON, 1982), and vitellogenin appears to bind thyroid hormones (MITCHELL and STILES, 1985). However, because of small quantities of T4-binding to vitellogenin (DAVISON *et al.*, 1978; MITCHELL and STILES, 1985; COOKSON *et al.*, 1988), it is unlikely that an increase in vitellogenin is responsible for the enhanced thyrotropic activity in the E_2 -treated chick embryo. However, these possibilities should be examined in further experiments.

In the present study T4 levels in female embryos were significantly higher than in males. Although causes underlying this sex difference are unknown at present, it is possible that the sex difference is due partly to the significantly higher level of total plasma proteins including albumin in female embryos (Table 1).

The electrophoretic separation of T4-binding proteins in the present study was carried out at pH 8.6, which is somewhat above the physiological range of chicken

Fig. 1. Distribution of ^{125}I -thyroxine and serum proteins stained with Coomassie brilliant blue G-250 after electrophoresis in 19-day-old chick embryos.

Fig. 2. Distribution of ^{125}I -thyroxine and serum proteins stained with Coomassie brilliant blue G-250 after electrophoresis in 6-week-old chickens.

blood. However, there was no substantial difference in electrophoretic profiles from those separated under the physiological pH (pH 7.4) (DAVISON *et al.*, 1978; MITCHELL and STILES, 1985); this may indicate the validity of the present procedure to estimate T4 situation in circulating blood. The T4-binding profile of embryonic plasma was similar to that of growing chicken plasma. Although there is no report on T4-binding proteins in the chick embryo, the present result indicates the establishment of thyroid function and its transporting system before hatch, in addition to the maturation of hypothalamo-adenohypophyseal axis (THOMMES, 1987).

In conclusion, because of a possible correlation between plasma T4 and albumin, it would be advantageous to measure albumin concentrations when thyrotropic activity in the chick embryo is investigated.

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