Thyrotropic Activity of Estradiol in the Chick Embryo Develops without Increase in Pituitary Thyrotropin Releasing Hormone Binding

Leo M. HUYBRECHTS², Sadao HOSHINO¹, Masaaki WAKITA¹,
Yasuo KOBAYASHI¹, Veerle M. DARRAS², Eddy DECUYPERE³
and Eduard R. KÜHN²

¹ Faculty of Bioresources, Mie University, Tsu 514, Japan

² Laboratory of Comparative Endocrinology, K.U. Leuven,
Naamsestraat 61, B-3000 Leuven, Belgium

³ Laboratory for Physiology of Domestic Animals, K.U. Leuven,
B-3030 Heverlee, Belgium

The influence of 17β -estradiol (E_2) injections on plasma thyroid hormones and growth hormone (GH) and on pituitary thyrotropin-releasing hormone (TRH) binding was studied in 19-day-old chick embryos. The E_2 injection raised plasma levels of thyroxine (T_4) and triiodothyronine (T_3) without increases in the peripheral 5'-monodeiodination (5'-D) and the T_3 to T_4 ratio. The injection had no significant effect on plasma GH concentrations. Pituitary TRH binding was not affected by E_2 -treatment, suggesting that as contrasted with findings in mammals, changes in pituitary TRH receptor levels may not be responsible for the thyrotropic action of E_2 in the chick embryo.

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Key words: estrogen, chick embryo, TRH binding, thyroid hormone, growth hormone

Introduction

In the previous study we have demonstrated that plasma levels of thyroid hormones, together with hepatic 5'-monodeiodination (5'-D), rise by the administration of 17β -estradiol (E_2) into an allantonic blood vessel in 20-day-old chick embryos¹⁾. The mechanism by which estrogen elevates thyrotropic and peripheral 5'-D activities in embryonic chickens, however, remains obscure.

There is clear evidence that in the developing chick embryo, the hypothalamo-adenohypophyseal-thyroid axis matures until the days 10.5-11.5 of the embryonic period²⁾. The presence of thyrotropin-releasing hormone (TRH) receptors has been found in both pituitaries and brains of avian species including the chicken³⁾. Chicken pituitaries primed with E_2 were found to be more responsive to the prolactin-stimulating effects of hypothalamic extract and TRH⁴⁾. In mammals, it is well documented that estrogen stimulates thyrotropin (TSH) secretion⁵⁾ by modulating

pituitary TRH receptor levels⁶¹.

In view of these studies, it appeared of interest to examine whether the elevated plasma levels of thyroid hormones in the chick embryo treated with E₂ were due to a possible alteration in pituitary TRH binding.

Materials and Methods

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. At day 18 and 19 of incubation 80 embryos received an injection of 17 β -estradiol (E₂, Sigma) (1.5 μ g/embryo/day, n = 40) into an allantoic blood vessel or the same saline solution as that in which E₂ was dissolved (0.9% NaCl, n = 40). E₂ was dissolved in absolute ethanol and the solution was diluted 100-fold with 0.9% saline containing 0.1% bovine serum albumin. Four h after the last injection of E₂ or saline, blood samples were obtained by heart puncture and pituitaries were dissected out. Plasma samples were stored at -20°C until assayed.

³H-TRH (L-proline-2, 3, 4, 5-³H (N)-labelled, 119.2 Ci/m mol, NEN Res. Prod. USA) binding was measured according to the procedure described before⁶¹. Anterior pituitaries were rinsed in ice-cold 0.9% NaCl and pooled. Ten glands (7.1-10.0 mg) were homogenized in 150 μ l of TKM buffer (40 mM Tris, pH 7.4, 7.5 mM KCl, 2 mM MgCl₂) with a Teflon-glass homogenizer. For the assay, 30 μ l of fresh pituitary homogenate and 20 μ l of ³H-TRH (3 $\times$ 10⁻⁸M, 0.2 μ Ci) were incubated in the presence or absence of a 1000-fold excess of unlabelled TRH (Peptide Inst. Minoh, Osaka) for 40 min at 0°C. The reaction was stopped by the addition of 2 ml of cold TKM buffer and the bound fraction was immediately separated by suction through cellulose nitrate filters (pore size 0.45 μ m, Toyo Roshi, Japan) and rinsed 3 times with 2 ml of cold buffer. After drying in scintillation vials and addition of 10 ml toluene-based scintillator, radioactivity was measured in a Beckman LS-1800 scintillation counter. All assays were done in duplicate.

Plasma levels of thyroxine (T₄) and triiodothyronine (T₃) and the *in vitro* 5'-monodeiodination (5'-D) activity were measured as described before⁷¹. For 5'-D assay livers were homogenized in 2 ml of a phosphate buffer (0.15 M). After centrifugation 50 μ l of the supernatant was incubated for 1 h in a water bath at 37°C with 10 μ l of T₄ (4 μ M) and 250 μ l of dithiothreitol (3 mM). To stop the reaction 1 ml of ice cold Brij 35 (1.25%, Sigma) was added to each tube, followed by T₃ radioimmunoassay. Plasma growth hormone (GH) levels were measured in homologous GH radioimmunoassay using recently obtained monoclonal antibodies⁸¹.

All data were subjected to analysis of variance and the mean values were compared with Student's *t*-test or Cochran-Cox test.

Results

Plasma T₄ and T₃ levels of non-pipping embryos are shown in Table 1. Since most of the embryos had not perforated the air sac membrane, data on a few pipping embryos were omitted from the present results.

E₂ treatment significantly increased plasma T₄ concentration in embryos of both sexes. The increases in T₃ concentration were significant in females and pooled embryos, but not in males (Table 1).

Table 2 shows plasma T₃ to T₄ ratios and liver 5'-D activities in the non-pipping embryos. In females the T₃/T₄ ratio was not influenced by E₂ injection because of the parallel changes in T₄ and T₃ concentrations, but in males the increased T₄ concentration and unchanged T₃ concentration after E₂ treatment resulted in a significant decrease in the T₃/T₄ ratio. Liver 5'-D activities following E₂ treatment were not affected in all embryos (Table 2).

Plasma GH levels and ³H-TRH binding to pituitary are shown in Table 3. Plasma GH concentrations following E₂ administration showed slight increases in male and female embryos, though the increases were not significant (Table 3).

The total binding of ³H-TRH to pituitary homogenate was only 0.27% of added ³H-TRH. Specific binding of ³H-TRH to the pituitary, calculated by subtraction of

Table 1. Effect of estradiol (E₂, 1.5 µg/embryo/day, twice) injection on plasma thyroid hormone levels(ng/ml) in 19-day-old embryonic chickens

	T ₄ (ng/ml)		T ₃ (ng/ml)	
	Saline	E ₂	Saline	E ₂
Female	6.35±0.58(19)	10.03±0.86(15)**	0.262±0.019(19)	0.371±0.025(15)**
Male	6.50±0.42(16)	8.50±0.67(15)*	0.319±0.016(16)	0.320±0.022(15)
Pooled	6.42±0.36(35)	9.26±0.56(30)***	0.288±0.013(35)	0.345±0.017(30)*

Means±SE are shown with number of animals in parenthesis.

*P<0.05, **P<0.01, ***P<0.001 vs. saline control.

Table 2. Effect of estradiol (E₂, 1.5 µg/embryo/day, twice) injection on the ratio of plasma thyroid hormone levels (T₃/T₄) and liver 5'-monodeiodination (5'-D) activity in 19-day-old embryonic chickens

	T ₃ /T ₄ ×100		5'-D activity(T ₃ ng/mg protein)	
	Saline	E ₂	Saline	E ₂
Female	4.29±0.28(19)	3.84±0.23(15)	0.22±0.03(21)	0.26±0.02(16)
Male	5.12±0.31(16)	3.92±0.24(15)**	0.27±0.03(17)	0.23±0.02(14)
Pooled	4.67±0.22(35)	3.88±0.17(30)**	0.24±0.02(38)	0.25±0.01(30)

Means±SE are shown with number of animals in parenthesis.

**P<0.01 vs. saline control.

Table 3. Effect of estradiol (E₂, 1.5 µg/embryo/day, twice) injection on plasma growth hormone (GH) levels(ng/ml) and on ³H-TRH binding to pituitary in 19-day-old embryonic chickens

	GH(ng/ml)			Specific binding of ³ H-TRH to pituitary (cpm/mg protein)
	Female	Male	Pooled	
Saline	4.68±0.30(19)	5.43±0.46(13)	4.98±0.26(32)	3777±370(4)
E ₂	5.46±0.32(14)	6.11±0.47(13)	5.77±0.28(27)	3704±182(4)

Means±SE are shown with number of animals or observations in parenthesis.

nonspecific binding from the total, was 49.3 (saline) and 51.0% (E_2) respectively, and there were no statistical differences in pituitary bound specific radioactivities between saline and E_2 -treated embryos (Table 3).

Discussion

The present study confirms our previous finding that E_2 has thyrotropic activity in embryonic chickens¹¹. The effect was more pronounced in female birds perhaps because of a high sensitivity to E_2 . The high T_3 levels in female embryos may coincide with raised T_4 levels which will allow more substrate supply to the 5'-D enzyme. From the results of 5'-D activity it is clear that no stimulation of the enzyme occurs and accordingly the increased T_3 levels must be the result of a high substrate availability. This is in contrast with previous results where E_2 was able to increase 5'-D activity¹¹. One must point out, however, that in those experiments the stimulatory effects were only clearly visible in 20-day-old pipping animals whereas in the present study 19-day-old non-pipping embryos were used. This may indicate that the effect of E_2 , either directly or through a GH release, changes during embryonic development.

The result on pituitary TRH binding suggests that the thyrotropic effect of E_2 in chick embryos is not caused by changes of TRH receptor levels, as contrasted strongly with the effect observed in rats⁶). TRH receptors in chicken pituitary are known to be very similar to those of mammalian species, but properties of pituitary TRH receptors in embryonic chicks are unknown yet, though evidence as to the maturation of hypothalamus-pituitary-thyroid axis during embryonic life²) suggests an early completion of the receptors. Moreover, pituitary TRH binding in growing chickens is also not affected by an E_2 treatment (unpublished observation). Thus it seems that in the chicken exogenous estrogens exert their action on sites other than the level of pituitary TRH receptors. However, more detailed analysis of TRH receptor binding, including affinity (K_a), density of binding sites and pharmacology for TRH analogs, is required for the clarification of E_2 effect on pituitary thyrotrophs and other cells.

There was a slight but no significant increase in plasma GH concentrations of E_2 -treated birds (Table 3). This may relate in part to E_2 effects on pituitary and hypothalamic activities. It has been shown clearly that GH⁹) and hypothalamic hormones that release GH stimulate peripheral 5'-D activity in the chick embryo¹⁰) and chicken¹¹) so that the T_3 to T_4 ratio of blood plasma elevates following administration of these hormones, whereas TSH is mainly thyrotropic in the chicken¹¹). Therefore, based on the peripheral 5'-D activity and T_3 to T_4 ratio in the present study, it is unlikely that E_2 greatly stimulates TRH release to elevate plasma GH. More work is required to elucidate the precise action of estrogen on thyroid hormone synthesis and release in the chick embryos, though the lack of reliable chicken TSH assay such as RIA will restrict the study.

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エストラダイオールの鶏胚での向甲状腺活性は下垂体の甲状腺刺激ホルモン放出ホルモンの結合増加なしに発現する

レオ, ヒューブリチ²・星野貞夫¹・脇田正彰¹・小林泰男¹
フィーレ, ダラス²・エディ, デキュペリ³・エドワード, キューン²

¹ 三重大学生物資源学部 津市 514

² レーベンカソリック大学比較内分泌学教室 B-3000 レーベン ベルギー

³ レーベンカソリック大学家畜生理学教室 B-3030 ヘベリー ベルギー

19日齢の鶏胚を用いて17- β エストラダイオール (E_2) の投与が血漿甲状腺ホルモン, 成長ホルモン, 甲状腺刺激ホルモン放出ホルモン (TRH) の下垂体への結合に及ぼす影響を研究した。 E_2 投与は末梢の5'-脱ヨウ素, トリヨードサイロニン (T_3): サイロキシン (T_4) 比を増加させることなく血漿の T_4 , T_3 レベルの増加をもたらした。血漿中の成長ホルモンレベルには影響がみられ

なかった。 E_2 投与は下垂体への TRH 結合に影響を与えなかったので, E_2 の向甲状腺作用は哺乳類で観察されているのとは異なり, 下垂体 TRH 受容体レベルの変化によるものでないと思われる。

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キーワード: エストロジェン, 鶏胚, TRH 結合, 甲状腺ホルモン, 成長ホルモン