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Original article

Evidence for chicken GH as the only hypophyseal factor responsible for the stimulation of hepatic 5'-monodeiodination activity in the chick embryo

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Summary — The influence of an intravenous injection of chicken growth hormone (cGH), a total chicken pars distalis (PD) extract, and a PD extract depleted of cGH by immunoadsorption was studied in the 18-d-old chick embryo. Plasma concentrations of triiodothyronine (T3), thyroxine (T4), and hepatic 5'-monodeiodination (5'-D) activity were measured. An injection of total PD extract raised plasma T3, T4, and 5'-D activity, whereas a PD extract depleted of GH only increased plasma T4. The amount of cGH present in the PD extracts, as measured by homologous cGH radioimmunoassay, increased T3 and raised liver 5'-D, but had no effect on plasma T4. The effect on liver 5'-D was more pronounced with cGH than with a total PD extract, whereas the effect on plasma T3 was somewhat less pronounced. It was concluded that cGH increased the peripheral conversion of T4 into T3 in the chick embryo, whereas a PD extract depleted of cGH was purely thyrotropic. The PD extract also seemed to have 5'-D-suppressing activity.

chicken — growth hormone — hepatic 5'-monodeiodination — stimulation

Résumé — Mise en évidence de l'hormone de croissance comme seul facteur hypophysaire stimulant la 5'-déiodase hépatique chez l'embryon de poulet. *L'effet d'une injection intraveineuse de l'hormone de croissance du poulet (cGH), d'un extrait total de la pars distalis de poulet (PD) et du même extrait mais dont la fraction cGH a été éliminée au préalable par immunoadsorption, a été étudié chez l'embryon de poulet âgé de 18 jours. Les concentrations plasmatiques en triiodothyronine (T3), en thyroxine (T4) ainsi que l'activité de la 5'-déiodase hépatique (5'-D) ont été mesurées. Une injection de l'extrait total de la PD élève le taux de T3 et de T4 dans le sang et stimule l'activité de la 5'-D hépatique. L'extrait de la PD ne contenant plus la fraction GH n'augmente que le taux de T4. La quantité de cGH présente dans les extraits de la PD, estimée au moyen d'un dosage radioimmunologique homologue, augmente le niveau de T3 ainsi que l'activité de la 5'-D mais n'a pas d'influence sur le taux de T4. A l'inverse du taux de T3 plasmatique, la 5'-D hépatique est plus stimulée après traitement avec la cGH qu'avec l'extrait total*

hypophysaire. Il apparaît donc que la cGH augmente le taux de conversion périphérique de T4 en T3 chez l'embryon de poulet tandis que l'extrait de la PD ne contenant plus de fraction cGH a une action exclusivement thyréotrope et semble inhiber l'activité de la 5'-D.

poulet — hormone de croissance — 5'-déiodase hépatique — stimulation

Introduction

Several hormone preparations are known to stimulate hepatic 5'-monodeiodination (5'-D) activity in the chick embryo. The administration of either glucocorticoids or adrenocorticotropic hormone (ACTH) (Decuypere *et al.*, 1983), ovine prolactin (Kühn *et al.*, 1983; Decuypere and Kühn, 1985), and ovine growth hormone (GH) (Kühn *et al.*, 1986) all increase the circulating concentration of triiodothyronine (T3), the T3-to-thyroxine (T4) ratio, and the activity of liver 5'-D. These effects can be mimicked by hypothalamic hormones which increase the endogenous release of chicken growth hormone (cGH) (Kühn *et al.*, 1988b).

Recently, a monoclonal immunoadsorbent was used in a one-step purification of cGH from a crude pituitary extract (Berghman *et al.*, 1988); a total cGH molecular population was obtained which contained oligomeres of cGH but also the glycosylated form previously described (Berghman *et al.*, 1987).

In the following study the influence of this preparation together with the effect of the cGH-depleted hypophyseal extract on thyroid hormone metabolism was studied.

Materials and Methods

Eggs of a commercial strain (Hisex; Euribrid, Aarschot, Belgium) were incubated at 37.5 °C in a forced-draught laboratory incubator (Petersime, Zulte, Belgium) with continuous lighting; they were turned once every 2 h at a 45° angle. All injections (vol. 0.1 ml) to the 18-d old embryos were performed in an allantoic vessel situated close to the shell membrane. Blood was taken by cardiac puncture in heparinized tubes 2 h after injection, and the liver was excised.

A commercial kit was used to measure thyroxine (T4) (RIA, PEG; Abbott Diagnostic Division, Antwerp), whereas T3 was assayed using an antiserum preparation of Mallinckrodt, (Dietzenbach, FRG). Hepatic 5'-monodeiodination (5'-D) activity was measured in an *in vitro* system using liver homogenates in the presence of 0.15 µm T4 and 2.4 mM dithiothreitol (Decuypere *et al.*, 1983).

Pituitaries were collected from broiler chicks in a processing company and snap-frozen in liquid nitrogen. The cGH preparation was obtained as described previously (Berghman *et al.*, 1988) by means of murine monoclonal antibody-based immunoaffinity chromatography.

Radioimmunoassay of cGH preparation

The characterization of the cGH preparation and the specificity of the monoclonal antibodies used to develop the assay have been reported elsewhere (Berghman *et al.*, 1988). Two µg of affinity-purified cGH were iodinated by the chloramine-T method of Greenwood *et al.* (1963). The iodinated hormone was purified by gel permeation, diluted 10-fold with a Tris-HCl buffer (0.1 mol/l) pH 7.6, containing 0.15 mol NaCl/l and 1% (wt/vol) bovine serum albumin (BSA), and stored in a freezer for further use. A small immunoadsorbent (Berghman *et al.*, 1988) was used for additional purification of the tracer when necessary *(i.e.*, from 2 wk after iodination). A high-affinity monoclonal antibody, designated IIG3B7, was selected for use in the assay. Starting from ascites fluid, final antibody dilutions of 1/3 x 10^6 were used, yielding a detection limit of 10 ng cGH/ml or 0.2 ng per tube. Statistical analysis was done by ANOVA and least-square differences of the means.

Results

In a first series of experiments, 0.1 ml of total pars distalis (PD) extract (1/10 and 1/50 PD containing, 16.7 and 3.4 µg, of cGH, respectively), purified cGH (equivalent to 1/8 and 1/40 PD, 13.7 and 2.7 µg, of cGH, respectively), and GH-depleted PD fractions (1/8 and 1/40 PD containing < 1 ng of GH) were injected. The results are summarized in Figs. 1 and 2.

An increase in plasma T3 concentrations occurred with both the concentrations of total PD extract and purified cGH (Fig. 1). The increase was lower when cGH alone was injected and did not seem to be dose dependent. Plasma T3 levels were not influenced when the cGH-depleted fractions were injected.

1/10 total PD and 1/8 cGH-depleted PD were able to increase plasma T4 concentrations (Fig. 1). All the other injections were negative in this regard.

An injection of total PD or pure cGH increased the T3-to-T4 ratio to the same level (Fig. 2). However, only an injection of 1/50 PD was effective in stimulating *in vitro* 5'-D activity, whereas 1/10 PD was not (Fig. 2). Moreover, this response was less than the stimulation of 5'-D found after injection of cGH.

In a second series of experiments, 0.15 PD, the amount of cGH present in this fraction (13.1 µg of GH), and the depleted PD fraction were injected. In an additional experiment, the cGH and cGH-depleted PD were recombined and also injected. The results are summarized in Table I.

Again, total pituitary extract and cGH increased plasma T3 concentration, and this response was less pronounced when cGH was injected alone. Recombination of cGH and the depleted PD was also effective in this regard.

Similar T4 increases were found after injection of 0.15 PD, the cGH-depleted PD, or the recombination of cGH and the depleted PD. However, cGH alone had no

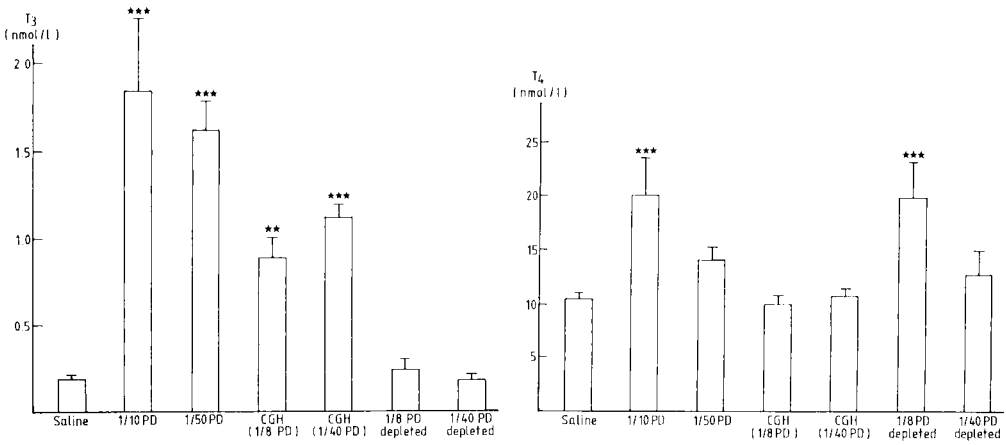

Fig. 1. Influence of saline, 1/10 PD, 1/50 PD, cGH found in 1/8 PD and 1/40 PD, and the cGH-depleted PD on plasma concentrations of T3 and T4 (nmol/l) 2 h after injection into 18-d old chick embryos. Mean ± SEM; $N = 10$; ** $P < 0.01$; *** $P < 0.001$ compared to control group (ANOVA).

effect in this regard. Comparable increases in the T3-to-T4 ratio were found after injection of 0.15 PD or cGH. A cGH-depleted PD did not increase the T3/T4 ratio but recombination of cGH with this depleted PD was again effective, although to a lesser extent than cGH alone ($P < 0.01$).

As in the first experiment, cGH seemed to be more effective in increasing hepatic 5'-D activity than the total PD extract, whereas recombination of cGH with the cGH-depleted PD resulted in partial inactivation of this stimulatory effect on 5'-D.

Discussion

For the first time, these results give direct evidence that cGH increases hepatic 5'-D and raises plasma concentrations of T3, but not of T4, in the chick embryo. Moreover, a hypophyseal extract devoid of this cGH did not raise plasma T3 or liver 5'-D in the concentrations we used, indicating that any other hormone present in the hypophysis of growing broiler chicks may not have this activity. The only other report in which cGH elevated plasma concentrations of T3, but not of T4, was a study of adult chickens by Scanes *et al.* (1986). The present study therefore confirms the hypothesis that cGH, and not chicken prolactin, stimulates 5'-D in the chick embryo, since hypothalamic hormones which release cGH but not prolactin are also effective in this regard (Kühn *et al.*, 1988a, b).

Other observations may be of interest :

(1) No log dose-response was obtained after injection of cGH with regard to plasma T3 or liver 5'-D. This observation has already been reported in the chick embryo by Kühn *et al.* (1988a). Perhaps a trigger effect of a very sensitive system may be obtained.

(2) The influence of cGH on plasma T3 concentrations was less pronounced than the effect of an injection of hypophyseal extract, whereas its effect on hepatic 5'-D was more pronounced. This observation was true in both experiments. It is possible that the activation of 5'-D after total PD extract was decreased by an

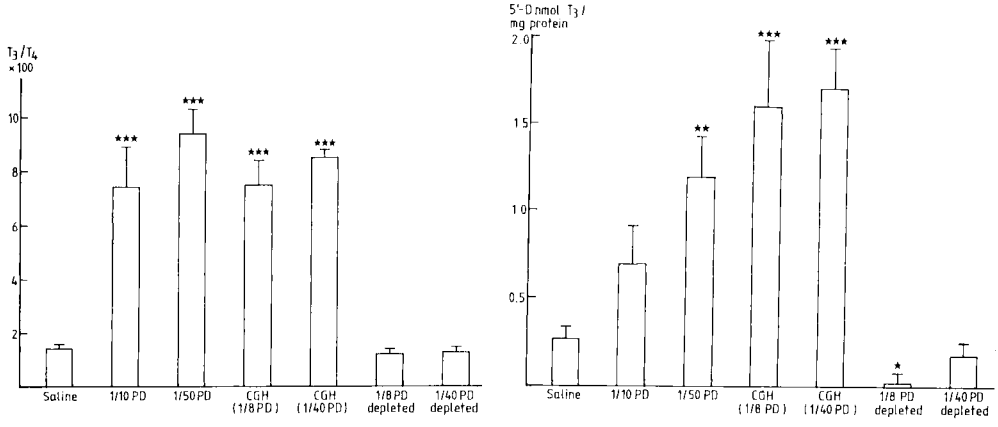

Fig. 2. Influence of saline 1/10 PD, 1/50 PD, cGH found in 1/8 PD and 1/40 PD, and the cGH-depleted PD on the T3/T4 ratio and hepatic 5'-D activity (nmol T3/mg protein). Mean ± SEM; $N = 10$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to control group (ANOVA).

Table I. Influence of saline, 0.15 PD, cGH, the cGH-depleted PD fraction and the recombination of this fraction with cGH on plasma concentrations of T3 and T4 (nmol/l), the T3 to T4 ratio (%) and the hepatic 5'-D activity (nmol T3/mg protein).

	Saline	*0.15 PD (13.8 µg cGH)*	*cGH (13.1 µg cGH)*	*0.15 PD-cGH*	*(0.15 PD-cGH) + cGH*
T3	0.386 ± 0.005 ^{d}	7.693 ± 0.757 ^{a}	2.668 ± 0.235 ^{b}	1.680 ± 0.371 ^{bd}	5.569 ± 1.200 ^{c}
T4	15.2 ± 1.8 ^{b}	37.2 ± 5.4 ^{a}	9.3 ± 0.4 ^{b}	35.9 ± 6.8 ^{ac}	34.3 ± 6.5 ^{ad}
T3/T4	2.92 ± 0.21 ^{c}	19.72 ± 2.26 ^{ae}	24.27 ± 2.11 ^{ab}	3.79 ± 0.51 ^{c}	14.11 ± 3.21 ^{de}
5'-D	0.23 ± 0.06 ^{c}	1.95 ± 0.27 ^{a}	3.90 ± 0.50 ^{b}	0.42 ± 0.05 ^{cd}	1.09 ± 0.23 ^{d}

Results were determined 2 h after injection into 18-d-old embryos. Mean ± SEM; $N = 10$. Means with different superscripts are significantly different ($P < 0.05$) by ANOVA and least-square differences.

unknown hypophyseal component. The observation of an extrathyroidal inhibitor of peripheral T4-to-T3 conversion in starved rabbits may be relevant in this regard (Nowak, 1987). This, however, does not explain why plasma concentrations of T3 increased more after an injection of total PD than the equivalent of cGH in this preparation. The observed increase in T4 after total PD extract injection, as substrate for conversion into T3 or better T3 transport into the blood, may be a possible explanation. It was shown that a T4 injection, when administered simultaneously with ovine GH into 18-d old chick embryos, had no effect on GH-stimulated 5'-D or plasma T3 (Kühn *et al.*, 1988b). In the present study, however, T4 was released by the thyrotropic activity of a PD extract. This may account for the differing results.

The observation that cGH-depleted PD extract resulted only in a T4 increase, without significant 5'-D change, confirms previous observations made in the chick embryo or adult chicken that thyroid-stimulating hormone (TSH), which is present in these extracts, is purely thyrotropic and does not stimulate hepatic 5'-D by increasing the T4 substrate (Kühn *et al.*, 1988a, b).

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