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Original article

Impaired peripheral T_3 production but normal induced thyroid hormone secretion in the sex-linked dwarf chick embryo

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Summary — Plasma concentrations of thyroxine (T_4), triiodothyronine (T_3) and chicken GH (cGH), together with hepatic 5'-monodeiodination (5'-D) activity, were measured in normal (Dw) and dwarf chick (dw) embryos at incubation d 18. An injection of 10 μg of ovine GH (oGH) raised plasma concentrations of T_3 in Dw embryos after 1 and 2 h and stimulated hepatic 5'-D activity after 2 h. A non-specific increase in T_4 was also observed after 1 h in Dw animals probably due to the heterologous nature of the injection. These effects were not observed in dw embryos. An injection of 1 μg of TRH was able to increase cGH levels after 15 min in Dw embryos, whereas the observed increase in the dw group was not significant. In Dw embryos, 0.01, 0.1 and 1 μg of TRH increased plasma concentrations of T_3 in a dose-dependent way, whereas in dw embryos, no reaction to the TRH injections was seen, except for the highest dose used. Contrary to this observation, T_4 was increased to the same level in both Dw and dw embryos following TRH injections. An injection of 1 μg of ovine CRH increased corticosterone after 0.5 h and elevated T_3 and T_4 after 2 h to the same extent in Dw and dw embryos. It is concluded that the thyrotrophic activities of TRH and oCRH and the corticotropic activity of oCRH do not differ between normal and sex-linked dwarf embryos. However TRH and GH were unable to stimulate the T_4–T_3 conversion in the liver of dw embryos, presumably due to the lack of hepatic GH receptors in these animals.

chicken / dwarf / thyroid / TRH / CRH / GH

Résumé — Inhibition de la production périphérique de la triiodothyronine avec une stimulation normale de la sécrétion des hormones thyroïdiennes chez les embryons de poulet nain dont le gène de nanisme est lié au sexe. *Les concentrations plasmatiques de thyroxine (T_4), triiodothyronine (T_3), d'hormone de croissance (cGH) ainsi que l'activité de désiodation hépatique de la T_4 (5'-D) ont été mesurées chez les embryons de poulet normaux (Dw) et nains (dw) au 18e jour de l'incubation. L'injection de 10 μg de GH ovine (oGH) augmente les taux plasmatiques de T_3 chez les embryons Dw après 1 et 2 h et stimule la 5'-D après 2 h. Une h après l'injection de oGH, on observe également une augmentation non spécifique de T_4 chez les animaux Dw. Cette augmentation est probablement due au caractère hétérologue de l'injection. Elle n'a aucun effet chez les embryons dw. Une injection de 1 μg de TRH stimule la libération de cGH après 15' chez les embryons Dw,*

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alors que l'effet n'est pas significatif chez les embryons dw. L'augmentation du taux de T_3 observée après injection de 0,01, 0,1 et 1 µg de TRH chez les embryons Dw dépend de la dose injectée; chez les embryons dw, seule la plus forte dose de TRH induit une faible augmentation de la teneur en T_3. Par contre, une injection de TRH augmente de manière comparable la concentration de T_4 dans le sang chez les embryons Dw et dw. Une injection de 1 µg de CRH induit chez les 2 types d'embryons, une augmentation de la corticostérone après 0,5 h et de T_3 et T_4 2 h après l'injection. Il en est déduit que les activités corticotrope de oCRF et thyréotrope de CRF et de TRH ne sont pas différentes chez les embryons Dw et dw. Seule la 5'-D hépatique, stimulée chez les embryons Dw par la TRH et la GH, ne l'est pas chez les embryons dw, probablement en raison d'une déficience en récepteurs hépatiques de la GH chez ces animaux.

poulet / nanisme / thyroide / TRH / CRH / GH

INTRODUCTION

Growth inhibition in the sex-linked dwarf chicken is not due to low growth hormone (GH) levels (which may be even higher after hatching), but probably to reduced plasma concentrations of insulin-like growth factor I (IGF I) and triiodothyronine (T_3) (Scanes *et al,* 1983; Huybrechts *et al,* 1987).

However, in the chick embryo prior to pipping, the presence of the dwarf gene does not result in a changed plasma concentration of T_3, thyroxine (T_4) or IGF I and II, or a changed T_4 liver 5'-monodeiodination activity (5'-D) (Huybrechts *et al,* 1989). The well known increase in plasma concentrations of T_3 (Decuypere *et al,* 1979) is less pronounced in dwarfs only after perforation of the air sac membrane, whereas liver 5'-D activity is lower (Huybrechts *et al,* 1989), indicating that the dwarfing gene may be related to a deficiency in the process of T_4 to T_3 conversion.

In chick embryos, GH, or a hypothalamic hormone that releases GH, stimulates hepatic 5'-D activity prior to pipping and profoundly increases plasma concentrations of T_3 without affecting T_4 (Kühn *et al,* 1988 a, b; Berghman *et al,* 1989).

Previous results have shown that this effect is not present in sex-linked dwarf embryos following injection of ovine GH (Kühn *et al,* 1986). In the present study, this effect was reinvestigated in dwarfs using the thyrotrophin releasing hormone tripeptide (TRH), which is known to be a powerful secretagogue of GH in the growing chick (Scanes *et al,* 1986), and is also effective in the chick embryo (Kühn *et al,* 1988b).

Since CRH is known to have a thyrotrophic effect in the chick embryo, but not a somatotrophic effect (Meeuwis *et al,* 1989), the influence of CRH on hepatic 5'-D, T_3 and T_4 levels in chick embryos of the dwarf and normal genotypes was also included in this study.

MATERIALS AND METHODS

The eggs used in these experiments came from the mating of heterozygous sires (Dwdw) with dwarf females (dw-) at the Laboratoire de Génétique Factorielle, INRA, Jouy-en-Josas, France. In order to make a distinction between control and dwarf chick embryos, the dwarf (dw) gene was linked with the silver gene(s) for down colour. This resulted in brown-feathered dwarf and white-feathered normal embryos. The gene for down colour did not interfere with the hormonal parameters studied. The eggs were incubated at

37.5 °C in a forced-draught incubator with constant light, and a relative humidity of 70%.

All injections were made at d 18 of incubation into a blood vessel situated close to the shell wall, and blood samples were taken by heart puncture after 15 min, 1 h and 2 h. Previous unpublished experiments indicate that effects are no longer present 4 h after the TRH or GH injection. Liver samples were stored at – 20 °C before assay for 5'-D, activity, as described previously (Darras *et al,* 1980). The injections consisted of oGH (NIAMDD-oGH-13), TRH and ovine CRH (UCB Bioproducts, Brussels, Belgium).

T_3 and T_4 concentrations in plasma were assayed using tracer obtained from Amersham International (UK), rabbit T_3 antiserum from Mallinckrodt (GFR) and laboratory-raised rabbit T_4 antiserum (Jacobs *et al,* 1988). This T_4 antiserum did have a 3.5% cross-reactivity with T_3. Intraassay variation for T_4 was 2.5% and interassay variation was 17.2%.

T_4 was detectable at concentrations as low as 3.12 ng/ml. The interassay variation of the T_3 assay was 2.9%, the intraassay variation was 6.2% and the detection limit was 125 pg/ml. The cross reactivity of the T_3 antiserum with T_4 was 0.3%.

The radioimmunoassay for cGH was developed using a high affinity murine monoclonal antibody (Berghman *et al,* 1987). Briefly, 20 µl of plasma or standard solution (2-200 ng/ml) were incubated for 24 h at room temperature with 100 µl of tracer (^{125}I-cGH prepared following the iodogen method, 8000 cpm in 0.5% pre-immune mouse serum) and 100 µl of monoclonal antibody (ascites 1/5.10^6). The next day, 100 µl of goat-antimouse antiserum (1/40) was added for another 24-h incubation. After centrifugation at 2400 x g for 10 min, the supernatant was aspirated and the precipitate was counted in a gamma-counter. The detection limit of the system was 2 ng/ml and there was no cross-reactivity with other pituitary hormones. Intraassay variability was 4.0% and interassay variability 15.5%. Chicken plasma dilution test and the loading test showed good parallelism with the standard curve.

Before assaying with a commercial RIA kit for corticosterone (Cambridge Medical Diagnostics, UK), 200 µl of plasma were extracted with 1 ml of dichloromethane and concentrated. Recovery with the extraction method was about 95%. Cross-reactivity of this kit was 0.6% for progesterone, 0.4% for cortisol, 0.2% for cortisone, and less than 0.2% for all other normally occurring steroids. The detection limit of the assay was 1.95 ng/ml, intraassay variation was 3.7%, and interassay variation was 5.2%.

Statistical analysis between groups was made by analysis of variance (ANOVA), followed by a least-squares difference significance test when F was significant.

RESULTS

Injection of oGH (fig 1)

An injection of 10 µg of oGH in 18-d old Dw chick embryo, raised plasma concentrations of T_3 after 1 and 2 h. This increase was more pronounced after 2 h when hepatic 5'-D activity was also stimulated and the plasma T_3 to T_4 ratio was raised. No increase in T_3 plasma levels or stimulation of 5'-D activity was observed in dw embryos following injection of 10 µg of GH.

The injection of oGH also increased plasma T_4 after 1 h in Dw, whereas the increase in dw was nearly significant. No effect on T_4 was seen 2 h following injection of GH.

Injections of TRH (fig 2)

TRH was injected at doses of 0.01, 0.1 and 1 µg, and blood samples were taken at 15 min and 2 h following injection.

Only the highest dose of TRH was able to increase circulating GH levels from control levels of 5.4 ± 0.2 to 10.6 ± 2.2 ng/ml after 15 min in Dw embryos. Control levels of dw embryos (6.6 ± 0.3 ng/ml) were not raised significantly 15 min after injection of 1 µg of TRH (8.8 ± 1.8 ng/ml). Two h after

the injection, all levels were similar to those of the control groups.

Plasma concentrations of T_4 after 2 h were increased equally in dw and Dw embryos for each dose of TRH used, and this effect was dose-dependent. No effect was present after 15 min (fig 2). In Dw embryos, every dose of TRH increased plasma concentrations of T_3 in a dose-dependent way; the highest dose resulting in a more than 5-fold increase. In dw embryos, however, no reaction to the TRH injections was seen except for the highest dose used, where a small increase in T_3 was present (fig 2).

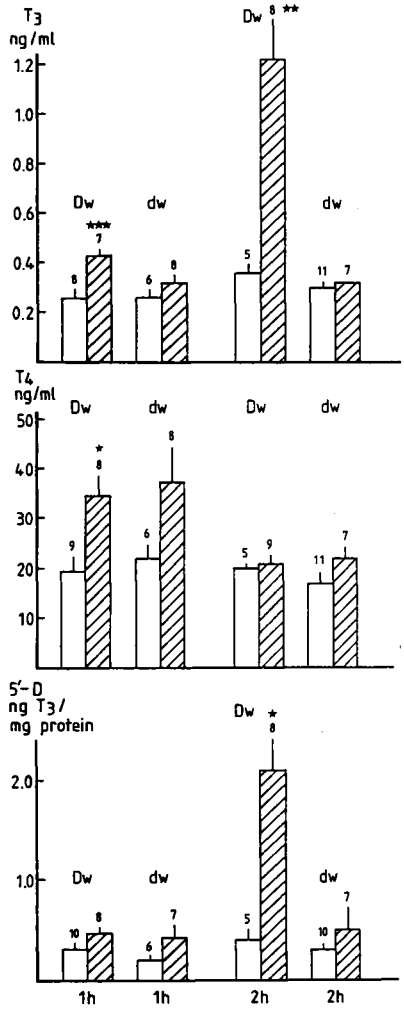

Fig 1. Injections of saline (open bars) or 10 μg oGH (crossed bars) into 18-d-old normal (Dw) and dwarf (dw) chick embryos and their effect 1 and 2 h later on plasma concentrations of T_3, T_4 and the hepatic 5'-D activity. Mean ± SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by ANOVA. Number of animals are as indicated.

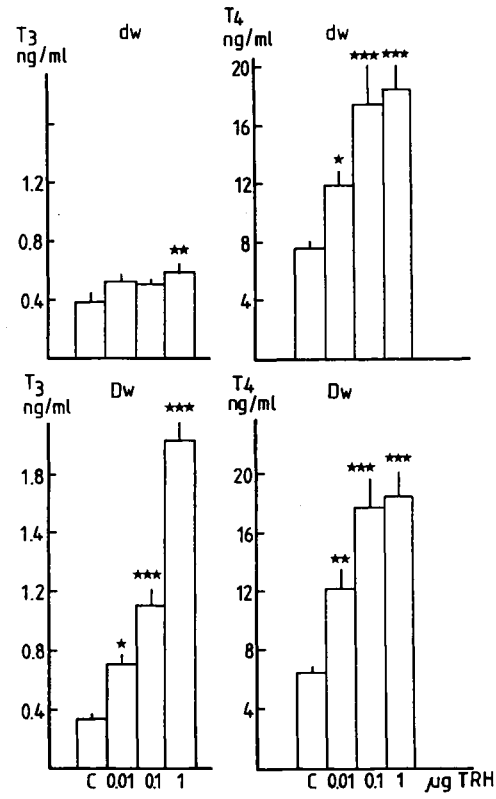

Fig 2. Injections of saline, 0.01, 0.1 and 1 μg TRH into 18-d-old Dw and dw chick embryos, and their effect 2 h later on plasma concentrations of T_3 and T_4. Mean ± SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by ANOVA. Number of animals are as indicated.

The hepatic 5'-D activity was only stimulated after 2 h when the highest TRH dose (1 μg) was used (table I) in Dw embryos. No stimulation was present in livers of dwarf chicks.

Injections of oCRH (fig 3)

Injections with 1 μg of oCRH were ineffective on circulating T_3 and T_4 concentrations in 18-d-old dwarf and normal embryos af-

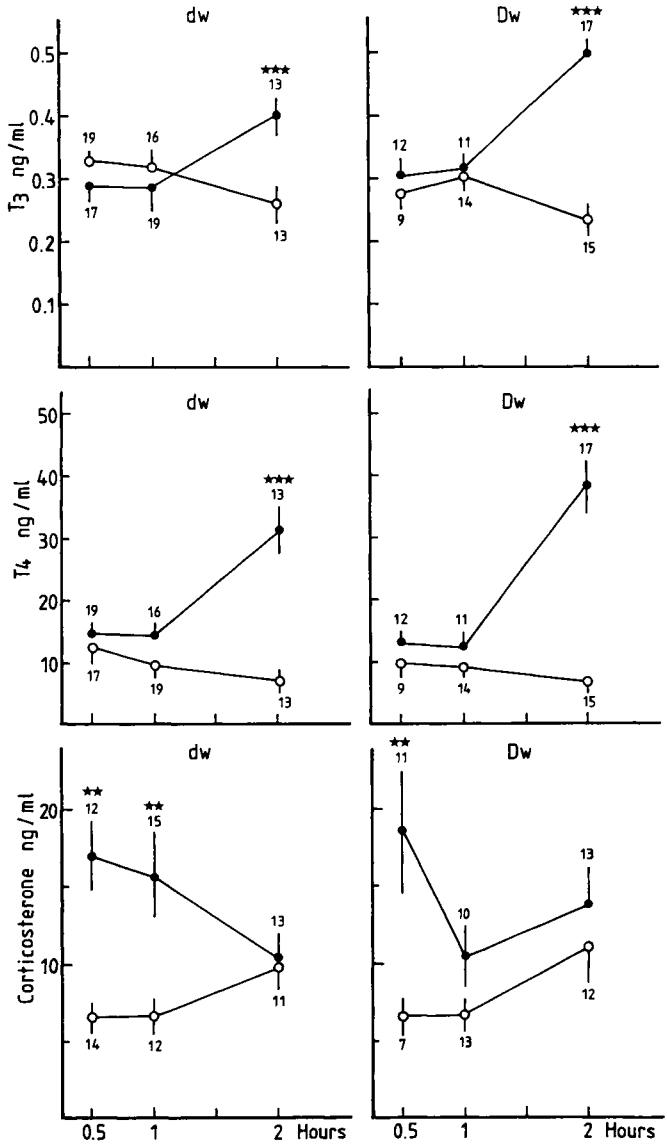

Fig 3. Injections of saline (o—o) and 1 μg of oCRH (●—●) on plasma concentrations of T_3, T_4 and corticosterone after 30 min, 1 h and 2 h. Mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by ANOVA. Number of animals are as indicated.

Table I. The influence of an injection of different doses of TRH into an allantoic blood vessel of 19-day-old chick embryos on the liver 5'-D activity after 2 h expressed as ng $T_3.h^{-1}.mg^{-1}$ protein. Mean ± SEM. * $P < 0.05$ compared to control by ANOVA.

	Control	*0.01 µg TRH*	*0.1 µg TRH*	*1 µg TRH*
dw	0.133 ± 0.025 (10)	0.136 ± 0.020 (12)	0.117 ± 0.021 (12)	0.126 ± 0.030 (11)
Dw	0.144 ± 0.020 (15)	0.115 ± 0.015 (12)	0.176 ± 0.031 (13)	0.227 ± 0.030* (12)

ter 0.5 and 1 h. However, 2 h after injection, CRH increased both T_3 and T_4 the latter in a far more pronounced way, resulting in a sharp decrease of the T_3 to T_4 ratio.

An injection of 1 µg of oCRH was effective in increasing plasma corticosterone concentrations after 0.5 h in normal and dwarf embryos. This effect remained present after 1 h in dw birds, but had disappeared in normal ones. Two h following injection of CRH, no significant corticosterone increases could be found in either group of animals.

DISCUSSION

These results confirm previous experiments on normal chick embryos, indicating that an injection of cGH (Berghman *et al,* 1989; Darras *et al,* 1990) or hypothalamic hormones, which are known to release GH (Kühn *et al,* 1988a, b), will increase plasma concentrations of T_3, and the T_3 to T_4, ratio by stimulating hepatic 5'-D activity. The sex-linked dwarf chicken embryo, however, was totally ineffective in this respect. Only the highest dose of TRH could increase plasma T_3 (x 1.6), but not hepatic 5'-D activity nor the plasma T_3 to T_4 ratio, which actually decreased. The effect on plasma T_3 was far below the one found in normal chick embryos (x 6.7), using the same dose of TRH.

Dwarf animals, however, could release T_4 from the thyroid gland, following TRH or oCRH stimulation. The injection of 1 µg of oCRH also increased plasma T_3 in dwarf (and normal) embryos, but actually decreased the T_3–T_4 ratio, as did 1 µg of TRH in dwarfs. The effect of 1 µg of TRH was even less pronounced than the effect of 1 µg of oCRH, indicating that on an equimolar base, oCRH is more than 14 times more potent than TRH in stimulating thyroid hormone secretion. Moreover, the dwarfing gene did not seem to affect a differential release of T_4 and T_3, since the decrease in the T_3–T_4 ratio, following oCRH stimulation, occurred in both normal and dwarf embryos. The chick embryo has a T_3–T_4 ratio in the thyroids, which equals the one in plasma (Decuypere *et al,* 1982), and is able to increase plasma concentrations of T_4, together with T_3, following TSH stimulation (Kühn *et al,* 1988a). This release resembles the one observed with a high dose of TRH in dwarf embryos and oCRH in normal and dwarf animals, since the T_3–T_4 plasma ratio also decreased without any influence on hepatic 5'-D following the injection of TSH. It seems, therefore, that any thyrotropic activity of TRH, oCRH or TSH will preferentially release T_4 in the chick embryo. However,

one cannot exclude the possibility of a substrate effect on T_3 formation. T_4 injections into 18-d-old normal embryos are able to induce a rise in T_3 after 15 min, but not after 1 or 2 h, and they have no effect on hepatic 5'-D activity (Kühn *et al,* 1988b).

We do not believe that the small increase in plasma T_4, 1 h after injection of oGH, has physiological significance, since in a previous study, an injection of hpGRF, which releases cGH, was without any effect on circulating levels of T_4 after 15 m, 1 or 2 h (Kühn *et al,* 1988b). Moreover, injections of cGH never increase plasma T_4 (Berghman *et al,* 1989) but may actually decrease it (Darras *et al,* 1990), due to a stimulated peripheral conversion into T_3. Heterologous injections of oGH into several fish species have also been shown to be thyrotropic and increase plasma concentrations of T_4 (Milne and Leatherland, 1978; Grau and Stetson, 1979; Byamungu *et al,* 1990).

The thyrotrophic and adrenocorticotrophic activity of oCRH, in this study, confirms a previous report using a Hisex White strain of chick embryos (Meeuwis *et al,* 1989). Both activities were equally present in dwarf and normal chick embryos. One may wonder whether these effects of oCRH are the result of a direct stimulation of thyrotrophin or due to mediation of ACTH and corticosterone release, which are known to increase plasma concentration of T_3 and T_4 in the chick embryo (Decuypere *et al,* 1983). The latter hypothesis may be more valid, since corticosterone release was already observed after 0.5 h, whereas T_3 and T_4 only increased after 2 h.

Only in Dw animals was TRH able to release GH significantly, though several individual data in dw chicks clearly point to an increased GH release. It is known that GH may be released in dwarf chick embryos following injection of hpGRF as a secretagogue (Huybrechts *et al,* 1985). Moreover, a stimulation in GH release, following administration of a hypothalamic factor, may not always be easy to demonstrate, as discussed by Lauterio and Scanes (1988), due to the episodic release pattern of GH (Buonomo *et al,* 1987). This may also explain why TRH was first claimed to have no effect on GH release in the chick embryo (Decuypere and Scanes, 1983), whereas later studies could raise GH in plasma of chick embryos 15 m and 2 h after TRH injection, using the same dose as in this study (Kühn *et al,* 1988a).

In conclusion, several hypothalamic hormones maintain a thyrotrophic control in the chick embryo carrying the dwarfing gene on the sex chromosome. However, they are unable to obtain high plasma T_3 concentrations, probably not because of a diminished GH release following TRH stimulation, which indeed may even be increased in growing chickens (Scanes *et al,* 1986). Several reports point to diminished hepatic GH-receptor binding in sex-linked dwarf chickens (Leung *et al,* 1987) or in adult dwarf broiler breeding hens (Kühn *et al,* 1989), and preliminary observations also indicate an impaired binding of GH to its hepatic receptor in dw embryos (Vanderpooten, unpublished observation). The lack of hepatic GH receptor in the dwarf chick embryo at the end of incubation therefore, seems to be responsible for the observed differences between dw and Dw embryos. At the same time, it indicates the importance of the development of liver GH receptors at the end of incubation for the maturation of thyroxine metabolism.

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