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Original article

A decreased capacity of hepatic growth hormone (GH) receptors and failure of thyrotrophin-releasing hormone to stimulate the peripheral conversion of thyroxine into triiodothyronine in sex-linked dwarf broiler hens

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Summary — The effect of two different doses of thyrotrophic releasing hormone (TRH) upon the plasma levels of growth (GH) and thyroid hormones in both sex-linked dwarf (dw) and normal (Dw) broiler hens was determined.

In normal hens, 1.5 and 24 µg TRH/kg increased the GH plasma concentrations after 15 min. Plasma concentrations of T3 increased significantly 1 h after TRH injection, whereas T4 concentration decreased after 2 following injection of 24 µg/kg TRH.

In dwarf hens both doses of TRH increased the plasma concentrations of GH and the GH response lasted longer. However, TRH was ineffective in raising T3 and T4 levels. Saline-injected dwarf birds showed no differences in plasma T4 and T3 levels in comparison with normal hens.

A smaller number of hepatic cGH receptors was found in dwarf hens, whereas the affinity of the hepatic GH receptor was not influenced by the genotype.

It is concluded that the sex-linked dwarf broiler hen is unable to respond to a TRH-induced GH stimulus probably because of a deficiency in hepatic GH receptors resulting in a failure to stimulate the T4 to T3 converting activity.

monodeiodination — TRH — thyroid hormones — GH receptors — dwarf broilers

Résumé — Diminution des récepteurs hépatiques à l'hormone de croissance et défaut de stimulation par TRH de la conversion de thyroxine en triiodothyronine chez les poules de chair naines. *L'effet de deux doses de TRH sur les concentrations plasmatiques de l'hormone de croissance (GH) et des hormones thyroïdiennes chez les poules de chair naines (dw) ou normales (Dw) a été déterminé. Chez les poules normales, 1,5 et 24 µg de TRH/kg poids corporel augmentent le taux plasmatique de GH après 15 min. La concentration de T3 augmente signficativement 1 h après l'injection de TRH tandis que celle de T4 diminue 2 h après injection de 24 µg TRH/kg. Chez les poules naines, les deux doses de TRH utilisées augmentent plus longtemps le taux plasmatique de GH que chez les poules normales mais sont sans effet sur les*

niveaux plasmatiques de T3 et T4. Ces derniers ne sont d'ailleurs pas différents de ceux observés chez les poules témoins naines ou normales injectées de sérum physiologique. Enfin, un nombre moindre de récepteurs hépatiques à cGH est trouvé chez les poules naines, alors que l'affinité de ces récepteurs n'est pas influencée par le génotype. On peut en conclure que les poules de chair naines (dw) ne peuvent pas répondre à une stimulation de GH induite par la TRH, vraisemblablement à cause d'une déficience en récepteurs hépatiques pour la GH et cela entraîne une incapacité à stimuler de désiodation de T4 en T3.

monodésiodation — TRH — hormone thyroïdienne — récepteur de GH — poule de chair naine

INTRODUCTION

In adult layer hens the hypothalamic thyrotrophic releasing hormone (TRH) decreases plasma concentrations of thyroxine (T4) and stimulates the peripheral conversion of T4 into triidothyronine (T3), as judged by increased plasma levels of T3 and a stimulated liver 5'-monodeiodination (5'-D) activity (Kühn *et al.*, 1988a). An injection of thyrotrophin (TSH), however, is purely thyrotropic and increases plasma concentrations of T4 (the principal iodohormone of the chicken thyroid gland), without influencing its peripheral conversion (Kühn *et al.*, 1988a). Peripheral conversion seems to be under the control of GH as injection of ovine GH (oGH) into layer hens also increases plasma concentration of T3 and decreases T4, together with a stimulation of the liver 5'-D activity (Kühn *et al.*, 1987). It has been consequently shown that chicken GH (cGH) is equally effective in this regard when injected into broiler hens (Scanes *et al.*, 1986).

Growth deficiency in the sex-linked dwarf chicken is not due to low GH levels. These chicks even have higher levels after hatching (Scanes *et al.*, 1983) but reduced plasma concentrations of immunoreactive-somatomedin C (Huybrechts *et al.*, 1985), whereas plasma concentrations of T3 are depressed and those of T4 are normal (Scanes *et al.*, 1983).

Hypothalamic hormones which release GH or cGH itself stimulate the peripheral conversion of T4 into T3 in the chick embryo (Kühn *et al.*, 1988b; Darras *et al.*, 1989). This effect is not present in the sex-linked dwarf embryo of layer hens and it was suggested that the dwarf growth of these hens may be at least partly a consequence of a failure in this maturation process of peripheral T4 metabolism (Kühn *et al.*, 1986). Moreover, a diminished hepatic GH-receptor binding has been demonstrated in sex-linked dwarf chickens (Leung *et al.*, 1987).

Adult broiler hens with the *dw* dwarfing gene are extensively used in poultry breeding (Guillaume, 1976). In this study the influence of the *dw* gene on GH release and hepatic GH receptors and the peripheral conversion of T4 into T3 upon TRH administration have been investigated in broiler breeders.

MATERIALS AND METHODS

Normal and sex-linked dwarf females of White Rock origin selected over a period of 20 y and used as the parental line for producing cross-bred broiler females were obtained from the Institut de Sélection Animale (ISA) at Châteaubourg (France). Dwarf hens weighed ≈ 2.5 kg

and were 45 weeks of age, whereas normal chicks weighed 3.5 kg and were 55 weeks of age.

Saline and 1.5 and 24 μg TRH/kg (UCB, Belgium) were injected into a wing vein and blood samples were taken in heparin from the contralateral vein before, then 15 min, 1 h and 2 h after injection. After 2 h livers were excized and immediately frozen on dry ice. Following centrifugation plasma samples were kept at – 20 °C.

Chicken GH (cGH) as purified from a crude pituitary extract using monoclonal antibodies was used for the homologous GH radio-immunoassay (RIA) (Berghman *et al.*, 1988). The T3 and T4 concentrations in plasma were assayed by using tracer obtained from Amersham International (UK), rabbit T3 antiserum from Mallinckrodt (GFR) and a laboratory-raised rabbit T4 antiserum. This T4 antiserum had a 0.16 % cross-reactivity with T3. All RIAs had good parallelism with plasma dilution curves and an intra-assay variability of < 5 %.

For the determination of the hepatic GH receptors, microsomal fractions from individual livers were prepared (Shiu, 1973) and stored at – 20 °C. The 100.000 g pellet was resuspend-ed in Tris—HCl assay buffer (25 mM, pH 7.5) containing 10 mM $CaCl_2$ and 0.5 % w/v BSA. A purified pituitary preparation of cGH was iodinated using the iodo-gen iodination reagent, as described by Fraker and Speck (1978).

Immediately before assay the membranes were $MgCl_2$-treated according to the method of Kelly *et al.* (1979). $MgCl_2$ treatment increases the specific binding per mg protein from 1.10 to 1.94 % of the total radioactivity added. Specific binding of cGH was determined by incubating 100 μl ^{125}I-cGH (35 x 10^3 cpm) with the membrane fractions (100 μl), either in the presence or in the absence of 100 ng/tube (100 μl) of unlabeled cGH. Non-specific binding in the membrane preparations tested was 12—14 % of the total radioactivity added. After incubation at room temperature for 18—20 h, 2 ml of cold assay buffer was added to each tube which was then centrifuged at 4 °C for 30 min at 3000 *g*. The pellets were washed with 1 ml of assay buffer and again centrifuged at 4 °C (30 min at 3000 *g*). This pellet was counted in the γ-counter (LKB-Packgamma II 1720).

Scatchard analyses were carried out by incubating a fixed amount of membrane preparations with a fixed amount of labeled hormone and increasing amounts of unlabeled hormone.

Statistical analyses of the results were performed by a *t*-test for paired data and analysis of variance followed by the least-squares difference when *F* was significant.

RESULTS

Control values

Control values of cGH, T3 and T4 following saline injection did not alter for the time-period studied. No differences between dwarf and normal broiler breeders were found for these hormonal parameters (Table I).

GH

An injection of TRH was able to increase plasma GH concentrations after 15 min in normal broiler breeders (Fig. 1). This increase was more pronounced at the high dose (24 μg/kg) used, but increased GH levels 1 h after injection were present in the lower dose ($P < 0.01$, paired *t*-test). In dwarf broiler breeders both doses of TRH increased plasma concentrations of GH and the GH response also showed the tendency to last longer in the dwarf birds (Fig. 1).

T4

No stimulatory effect of TRH on plasma T4 levels could be observed in normal

Table I. Control values of cGH, T3 and T4 (ng/ml) following injection of saline at 0 h. Mean ± SEM (n = 8) in dwarf (dw) and normal (Dw) broilers.

	0	*15'*	*1 h*	*2 h*
GH				
dw	20.1 ± 1.5	23.1 ± 2.0	24.4 ± 2.6	17.2 ± 0.9
Dw	17.2 ± 2.6	18.4 ± 1.0	18.7 ± 2.3	17.4 ± 1.4
T3				
dw	0.28 ± 0.05	0.30 ± 0.04	0.30 ± 0.02	0.30 ± 0.03
Dw	0.34 ± 0.04	0.35 ± 0.04	0.36 ± 0.05	0.33 ± 0.04
T4				
dw	13.9 ± 0.7	12.3 ± 0.7	12.0 ± 0.6	12.4 ± 1.1
Dw	10.1 ± 1.0	10.3 ± 0.6	10.3 ± 0.4	9.6 ± 0.5

chickens. On the contrary, levels tended to decrease after 2 h ($P < 0.001$ for 24 µg/kg). In dwarf broiler breeders, no decrease in plasma T4 levels was observed (Fig. 1).

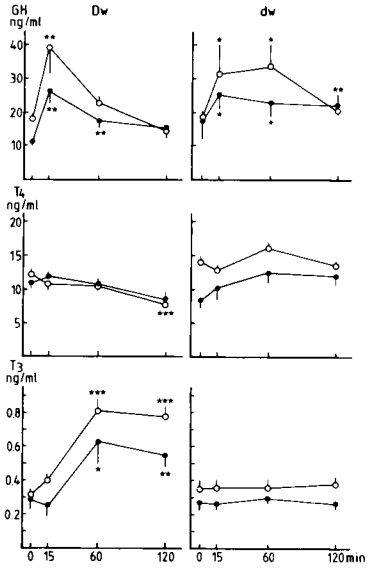

Fig. 1. Effect of an intravenous injection of TRH on plasma concentrations of GH, T3 and T4 (ng/ml) in adult normal (Dw) and sex-linked dwarf broiler breeders (dw). •—• 1.5 µg/kg; o—o 24 µg/kg TRH. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Paired *t*-test, $n = 8$ (mean ± SEM).

T3

An injection of 1.5 and 24 µg of TRH/kg resulted in marked increases in plasma concentrations of T3 in normal broiler breeders. These increases were more pronounced with the high doses, and persisted for at least up to 2 h following injection. However, no changes in circulating T3 concentrations were found in dwarf birds (Fig. 1).

Hepatic cGH receptors

Additions of 35 000 cpm of ^{125}I-cGH to the liver membrane fractions of normal broilers and dwarf hens resulted in the respective specific binding of 28.54 ± 1.58 % ($n = 7$) and 2.08 ± 0.54 % ($n = 7$) ($P < 0.001$) of the total counts added.

Binding affinity constant and binding capacity were determined by Scatchard analysis. Only 4 of the 7 dwarf hens had a high enough specific binding to allow this computation to be performed.

The affinity constants obtained were $3.40 \pm 0.52\ 10^9\ M^{-1}$ (normal, $n = 7$) and $4.91 \pm 0.81\ 10^9\ M^{-1}$ (dwarf, $n = 4$) and did not differ between both groups. The respective binding capacities were 14.67 ± 2.17 fmol/mg protein (normal, $n = 7$) *versus* 0.38 ± 0.06 fmol/mg protein (dwarf, $n = 4$). This difference in binding capacity between normal and dwarf hens is significant ($P < 0.001$) following analysis of variance.

DISCUSSION

The present study indicates that TRH is effective in releasing GH in adult normal and sex-linked dwarf broiler breeders, but that only in normal hens plasma concentrations of T3 are raised, presumably as a consequence of a stimulated peripheral 5'-D activity since at the TRH doses used

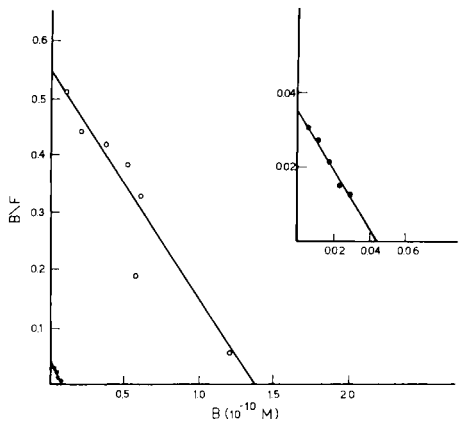

Fig. 2. Scatchard plot of normal (○) and dwarf (●) adult broilers (enlarged).

no thyrothropic activity could be found. The decrease in T4 concentrations observed after 2 h in normal hens but not in the sex-linked dwarfs may be the result of this increased deiodination.

The results on normal broiler breeders completely confirm our previous results on adult layers (Kühn *et al.*, 1988a) and suggest that a TRH-induced GH release is responsible for the observed 5'-D activation.

These results are, however, in contrast with a study by Hoshino *et al.* (1986) on 26-week-old Rhode Island Red chickens. Here an injection of 10 µg/kg TRH did not alter plasma concentrations of T3 or T4. In 11-week-old chicks, however, T3 increased whereas T4 remained unchanged.

Basal levels of T3 and T4 did not differ between normal and dwarf hens in our study, contrary to observations on high T4 and low T3 plasma concentrations in dwarf White Leghorn (Scanes *et al.*, 1983) or Rhode Island Red chickens (Hoshino *et al.*, 1986), which could indicate that in our adult dwarfing broiler breeders a normal 5'-D activity was present in peripheral tissue. The complete lack of T3 response to TRH therefore would probably be attributed to the failure of hepatic GH binding in dwarf hens. It may be noted that the GH is not able to stimulate the 5'-D activity and the T4 to T3 conversion in the liver of sex-linked dwarf embryos (Kühn *et al.*, 1986). A failure in hepatic GH binding in dwarf layers and a decrease in capacity in dwarf broilers (Leung *et al.*, 1987) has been observed. This decrease (from 11.23 to 6.04 fmol/mg) was, however, not as pronounced as in our study (from 14.67 to 0.38 fmol/mg). We therefore would like to state in conclusion that the deficiency in hepatic binding of GH as observed in the present study in the adult sex-linked dwarf broiler

breeder may explain the lack of effect of a GH-mediated stimulation of the 5'-D activity. It has been previously discussed that this lack of effect may also be related to the low IGF-I response after a GH-injection in dwarf chicks from a layer breed (Huybrechts *et al.*, 1988; Kühn *et al.*, 1989).

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REFERENCES

Berghman L.R., Van Beeumen J., Decuypere E., Kühn E.R. & Vandesande F. (1988) One-step purification of chicken growth hormone from a crude pituitary extract by use of a monoclonal immunoadsorbent. *J. Endocrinol.* 118, 381-387

Darras V.M., Huybrechts L.M., Berghman L., Kühn E.R. & Decuypere E. (1989) Ontogeny of the effect of purified chicken growth hormone on the liver 5'-monodeiodination activity in the chicken : reversal of the activity after hatching. *Gen. Comp. Endocrinol.* (in press)

Fraker P.J. & Speck J.C. (1978) Protein and cell-membrane iodinations with a sparingly soluble chloramide, 1, 3, 4, 6-tetrachloro-3A, 6A-diphenylglycoluril. *Biophys. Res. Commun.* 80, 849-857

Guillaume J. (1976) The dwarf gene *dw* : its effects on anatomy, physiology, nutrition, management. Its application in poultry industry. *World Poult. Sci.* J. 32, 285-301

Hoshino S., Suzuki M., Kakegawa T., Wakita M. & Kobayashi Y. (1986) Thyroid hormone response to thyrotrophin releasing hormone (TRH) in the sex-linked dwarf chicken. *Endocrinol. Jpn* 33, 675-682

Huybrechts L.M., King D.B., Lauterio T.J., Marsh J. & Scanes C.G. (1985) Plasma concentrations of somatomedin-C in hypophysectomized, dwarf and intact growing domestic fowl as determined by heterologous radioimmunoassay. *J. Endocrinol.* 104, 233-239

Huybrechts L.M., Michielsen R., Darras V.M., Berghman L.R., Decuypere E. & Kühn E.R. (1988) Effect of a methimazole induced hypothyroidism on a growth hormone induced insulin-like growth factor I response in normal and sex-linked dwarf domestic fowl. *Med. Sci. Res.* 16, 85-86

Kelly P.A., Leblanc G. & Djiane J. (1979) Estimation of total prolactin-binding sites after *in vitro* desaturation. *Endocrinology* 104, 1631-1638

Kühn E.R., Decuypere E., Huybrechts L.M. & Darras V.M. (1989) Hormonal control of the hepatic 5'-monodeiodination activity and its relation to the somatomedin production in the chicken. *In : Endocrinology of Birds. Molecular to Behavioral* (Wada M., Ishii S., Scanes C.G. eds), Springer-Verlag, Japan Scientific Societies Press (in Press)

Kühn E.R., Decuypere E., Iqbal A., Luysterborgh D. & Michielsen R. (1988a) Thyrotropic and peripheral activities of thyrotrophin and thyrotrophin-releasing hormone in the chick embryo and adult chicken. *Horm. Metabol. Res.* 20, 158-162

Kühn E.R., Huybrechts L.M., Decuypere E. & Mérat P. (1986) Endocrinological effects of the sex-linked dwarf gene III : prolactin and growth hormone fail to increase the liver T4-5'-monodeiodinase activity in the sex-linked dwarf embryo. *Proc. 7th Eur. Poult. Conf. Paris.* World Poultry Science Assoc., vol. 2, pp. 965-969

Kühn E.R., Vanderpooten A., Huybrechts L.M., Decuypere E., Darras V. & Sharp P.J. (1988b) Hypothalamic hormones that release growth hormone stimulate hepatic 5'-monodeiodination activity in the chick embryo. *J. Endocrinol.* 118, 233-236

Kühn E.R., Verheyen G., Chiasson R.B., Huts C., Huybrechts L., Van den Steen P. & Decuypere E. (1987) Growth hormone stimulates the peripheral conversion of thyroxine into triiodothyronine by increasing the liver 5'-monodeiodinase activity in the fasted and normal fed chicken. *Horm. Metab. Res.* 19, 304-308

Leung F.C., Stylens W.J., Rosenblum C.I., Lilburn M.S. & Marsh J.A. (1987) Diminished hepatic growth hormone receptor binding in sex-linked dwarf broiler and Leghorn chickens. *Proc. Soc. Exp. Biol. Med.* 184, 234-238

Scanes C.G., Marsh J., Decuypere E. & Rudas P. (1983) Abnormalities in the plasma concentrations of thyroxine, triiodothyronine and growth hormone in sex-linked dwarf and autosomal dwarf White Leghorn domestic fowl *(Gallus domesticus). J. Endocrinol.* 97, 127-135

Scanes C.G., Campbell R., Harvey S., King D., Malamed S. & Perez F. (1986) Growth hormone in birds : a comparative perspective. *In : Comparative Endocrinology. Developments and Directions* (Ralph D.L., ed.), Alan R. Liss Inc., New York, pp. 115-136

Shiu R.P.C. (1973) Radioreceptorassay for prolactin and other lactogenic hormones. *Science* 180, 968-971