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Original article

Thyrotropin-releasing hormone (TRH) is not thyrotropic but somatotropic in fed and starved adult chickens *

ER Kühn [1**], M Herremans [3], E Dewil [1], A Vanderpooten [1], P Rudas [2], T Bartha [2,3], G Verheyen [3], L Berghman [4], E Decuypere [3]

[1] *Catholic University of Leuven, Zoological Institute, Laboratory of Comparative Endocrinology, Naamsestraat 61, B-3000 Leuven;*
[2] *University of Veterinary Science, Department of Physiology, Budapest Pf 2, H-1400 Hungary;*
[3] *Catholic University of Leuven, Laboratory for Physiology of Domestic Animals, Kardinaal Mercierlaan 92, B-3030 Heverlee;*
[4] *Catholic University of Leuven, Laboratory for Neuroendocrinology and Immunological Biotechnology, Naamsestraat 59, B-3000 Leuven, Belgium*

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Summary — Adult fed and starved Warren chickens, 2 yr of age, and approaching the end of the second laying year, were injected iv with 1 of the following products: 10 μg of thyrotropin releasing hormone (TRH); 100 μg of bovine thyrotropin (bTSH); 100 μg of ovine growth hormone (oGH); saline. The influence on plasma concentrations of thyroxine (T_4), triiodothyronine (T_3) or chicken GH (cGH) were followed. Prior to injection, it was clear from the control values that starvation for 3 d decreased plasma levels of T_3 and increased cGH, whereas 7 d of fasting increased T_4 and cGH. The plasma levels of cGH were elevated > 10-fold at 15 min following the TRH challenge in food-deprived chickens compared to a < 4-fold increase in normal fed hens. This increase was followed by a rise in T_3 after 1 h, which was also more pronounced in the starved animals, whereas T_4 decreased or remained unaffected. Increases in T_4 can, however, be obtained with 100 μg TSH in normal fed (2-fold) or starved animals (> 3-fold). Following injection of 100 μg oGH, a significant increase in T_3 levels was observed which in fed animals was already present at 30 min, but the higher levels persisted for 1 and 2 h in fed and starved hens. At the same time, a decrease in T_4 was observed in both groups of GH-treated chickens. It is concluded that TRH at the dose used is not thyrotropic but has a somatotropic effect and is responsible for the peripheral conversion of T_4 into T_3.

TRH / thyrotropic / somatotropic / chicken

Résumé — La thyréolibérine (TRH) n'est pas thyréotrope mais somatotrope chez la poule adulte, nourrie ou à jeun. *Des poules Warren, nourries ou à jeun, âgées de 2 ans ou à la fin de leur deuxième année de ponte, ont reçu une injection intraveineuse de l'un des produits suivants :*

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10 μg de thyréolibérine synthétique (TRH), 100 μg d'hormone thyréotrope bovine (bTSH), 100 μg d'hormone somatotrope ovine (oGH) ou du serum physiologique. Les concentrations plasmatiques en thyroxine (T_4), triiodothyronine (T_3) et hormone de croissance (cGH) ont été étudiées à l'issue de ces traitements. Un jeûne de 3 jours a fait baisser le taux de base de T_3 circulante et augmenter celui de cGH, alors qu'un jeûne de 7 jours a élevé ces taux pour T_4 et cGH. Chez les poules à jeun, les niveaux plasmatiques de cGH ont été au moins 10 fois plus élevés 15 min après l'injection de TRH qu'après celle de serum physiologique; chez les animaux nourris, cette augmentation est restée inférieure à 4 fois. Dans tous les cas, la stimulation de cGH a été suivie d'une augmentation des taux sanguins de T_3, 1 h après l'injection – elle est aussi plus importante chez les poules à jeun – alors que T_4 a diminué ou n'a pas varié. Par contre, T_4 s'est élevée après l'injection de TSH aussi bien chez les poules nourries (x 2) que celles à jeun (x 3 ou plus). L'injection de oGH a provoqué une élévation significative des taux de T_3, visible dès 30 min chez les poules nourries; elle a persisté pendant 1 et 2 h chez les poules nourries et à jeun. Inversement, T_4 a diminué dans les deux groupes d'animaux soumis à ce traitement. En conclusion, TRH, aux doses utilisées, n'est pas thyréotrope mais somatotrope chez la poule adulte chez qui elle est responsable de la conversion périphérique de T_4 en T_3.

TRH / thyréotrope / somatotrope / poule

INTRODUCTION

In all vertebrates except fishes, the hypothalamic tripeptide thyrotropin-releasing-hormone (TRH) is known to release thyrotropin (TSH) from the hypophysis, which will affect both release and synthesis of triiodothyronine (T_3) and thyroxine (T_4). Consequently, plasma concentrations of these hormones are raised in response to TRH, though the major bulk of circulating T_3 is derived from peripheral monodeiodination of T_4 (Licht and Denver, 1990).

An injection of TRH in the chick embryo (Kühn *et al*, 1988) and in growing chickens (Michels *et al*, 1986) increases plasma concentrations of both T_4 and T_3. Although, at least for the chick embryo, the rise in T_3 may also be the result of a stimulated peripheral conversion of T_4 into T_3 by GH, which is released simultaneously with TSH in response to TRH (Scanes *et al*, 1985). However, in the adult chicken different doses of TRH increase plasma T_3 but not T_4, which may even be decreased (Kühn *et al*, 1988). This suggests that in adult chickens, although the thyroid is still responsive to TSH stimulation, TRH is not thyrotropic but only able to stimulate the peripheral conversion of T_4 into T_3 by its somatotropic effect.

This aspect of thyroid physiology has been reinvestigated in the following study on adult chickens in direct relation to the release of GH. Since food deprivation is known to increase the responsiveness of the hypophysis for GH release and to affect thyroid hormone levels (Harvey, 1990), the influence of food deprivation on plasma concentration of GH and thyroid hormones following TRH stimulation was also included in this study.

MATERIALS AND METHODS

Chickens used in these experiments were adult Warren SSL (2.5 kg, age 2 yr) approaching the end of the second laying year. They were housed under standard temperature (20 °C) and light conditions (16 L–8 D). Both fed and food-deprived hens (3 and 7 d) had free access to tap water. In the first experiment, saline and TRH (10 μg; UCB, Belgium), were injected into a wing vein and blood samples were taken from

this vein in heparin just before injections and after 15 min, 1 h and 2 h. This experiment was replicated (called exp 1i throughout the text). In the second experiment saline, ovine GH (100 µg; NIADDH, NIH, MD) and bovine TSH (100 µg; Sigma) was injected into a wing vein and blood samples were taken from the wing vein after 30 min, 1 h and 2 h.

Chicken GH (cGH) as purified from a crude pituitary extract using monoclonal antibodies was used for the homologous GH radioimmunoassay (RIA) (Berghman *et al*, 1988). The T_3 and T_4 concentrations in plasma were assayed by using tracer obtained from Amersham International (UK), rabbit T_3 antiserum from Mallinckrodt (Germany) and a laboratory-raised rabbit T_4 antiserum. This T_4 antiserum had a 0.16% cross-reactivity with T_3. All RIAs had good parallelism with plasma dilution curves, an intra-assay variability of < 5% and an interassay variability of < 10%.

Statistical analyses of the results were performed by a *t*-test for paired data, and analyses of variance and covariance with polynomials fitted through the repeated measurements (SAS, 1985).

RESULTS

Control values

Plasma concentrations of T_3, T_4 and cGH were not altered in fed and food-deprived chickens following saline injections (paired *t*-test) up to 2 h following injections. Pre-injection, basal control values for these hormones in fed and food-deprived chickens are summarized in table I. In both experiments, T_4 and cGH plasma concentrations were increased after 7 d of food deprivation, whereas T_3 was decreased in one experiment after 3 d and in the other after 7 d.

Table I. Control values for plasma concentrations of T_3, T_4 and cGH (ng/ml ± SEM) in normal fed and food deprived chickens.

	Normal fed	*3-d fasted*	*7-d fasted*
Experiment 1:			
T_3	0.84 ± 0.09	0.60 ± 0.078*	0.65 ± 0.07
(N)	(20)	(19)	(19)
T_4	7.28 ± 0.47	7.48 ± 0.61	8.97 ± 0.57*
(N)	(20)	(19)	(18)
cGH	3.76 ± 0.22	5.23 ± 0.49*	5.22 ± 0.56*
(N)	(20)	(20)	(19)
Experiment 2:			
T_3	1.14 ± 0.08		0.87 ± 0.05**
(N)	(30)		(30)
T_4	8.10 ± 0.66		12.33 ± 0.41***
(N)	(30)		(30)
cGH	2.97 ± 0.11		4.72 ± 0.63**
(N)	(30)		(30)

ANOVA * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to normal fed animals.

Postinjection values

TRH injection (exp 1 and 1i)

Following TRH injection plasma concentrations of cGH were increased after 15 min. The increase of cGH was much more pronounced in food-deprived (> 10-fold) compared to fed hens (< 4-fold) and resulted in a significant interaction between feed and treatment at 15 min and 1 h (table II). T_3 increased in normal fed and food-deprived animals after 1 and 2 h. The increase in T_3 after 2 h varied between 1.5-fold in normal fed chickens and up to 2.2-fold in chickens which had been food-deprived for 7 d. This difference is significant after adjusting for the zero time values by covariation. A small but significant decrease in T_4 was observed in fed and starved chickens 15 min and 2 h after TRH injections. Covariation for zero time level indicates that this decrease was more pronounced in chickens fed *ad libitum*.

In a repetition of the first experiment, comparable results were obtained (fig 1). However, the interaction between feed and treatment regarding T_3 increase was much more pronounced in exp 1i, even without covariation for zero time values ($P < 0.0001$). Two h after TRH injection the increase in T_3 was 2-fold in fed hens, but 3- and > 4-fold at 3 d and 7 d respectively in starved chickens. The observed decrease in T_4 following TRH administration was less pronounced in this instance, as a significant decrease was seen only in fed animals after 2 h. Basal cGH values did not differ between fed and starved chickens. There was no significant response of GH

Table II. Analysis of variance (with polynomials for repeated measurements). Influence of an injection of 10 μg TRH *versus* saline (= Treat) on the time dynamics of plasma concentrations of T_3, T_4 and GH in normal fed, and 3- and 7-d starved chickens (= Feed) (Exp 1).

	Main effects				*Interactions*		
	Feed (F)	*Treat (Tr)*	*Time (Ti)*	*F x Tr*	*F x Ti*	*Tr x Ti*	*F x Tr x Ti*
T_3 general	NS	<0.0001	<0.0001	NS	0.06	<0.0001	NS
Zero time	NS	NS	–	NS	–	–	–
15 min	NS	NS	–	NS	–	–	–
1 h	NS	<0.0001	–	NS	–	–	–
2 h	NS	<0.0001	–	NS	–	–	–
T_4 general	0.04	NS	<0.0001	NS	NS	0.003	NS
Zero time	NS	NS	–	NS	–	–	–
15 min	NS	NS	–	NS	–	–	–
1 h	0.03	NS	–	NS	–	–	–
2 h	NS	NS	–	NS	–	–	–
GH general	<0.0001	<0.0001	<0.0001	0.042	=0.003	<0.0001	=0.0014
Zero time	=0.022	NS	–	NS	–	–	–
15 min	=0.0002	<0.0001	–	=0.011	–	–	–
1 h	<0.0001	<0.0001	–	=0.0015	–	–	–
2 h	=0.014	NS	–	NS	–	–	–

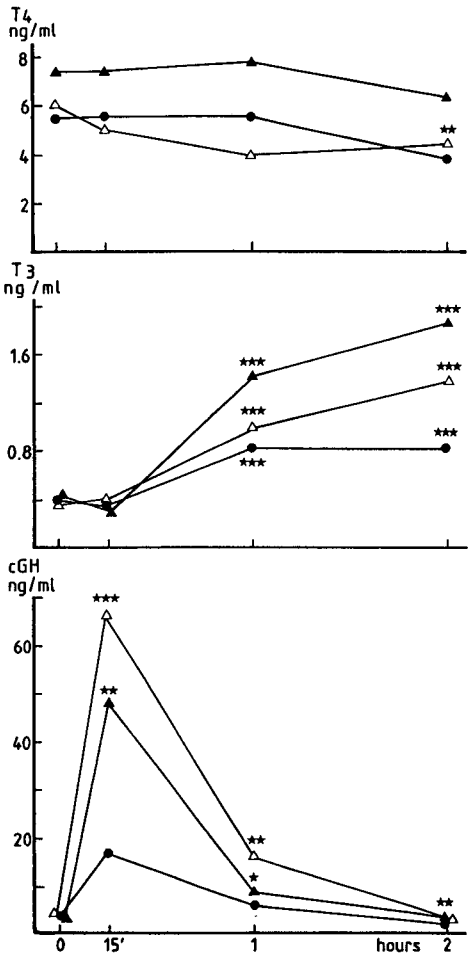

Fig 1. Influence of an injection at zero time of 10 μg TRH on plasma concentrations (ng) of T_3, T_4 and cGH in adult chickens (n = 10) normally fed (●) and food deprived chickens for 3 d (Δ) or 7 days (▲). $P < 0.05$; ** $P < 0.001$ paired t-test (exp 1i).

to TRH in fed animals. However, in starved chickens the cGH increase was 18-fold higher 15 min after TRH injection and remained significant for at least up to 1 h.

TSH and GH injection (fig 2, exp 2)

A significant increase in T_3 levels was observed following injection of 100 μg oGH which was evident as early as 30 min in fed animals and was maintained at 1 h and 2 h in fed and starved hens. Saline and TSH did not have any effect on T_3 in fed chickens. However, in starved hens TSH induced a small increase in T_3 after 1 and 2 h. A small decrease in T_4 after 2 h was observed in all GH-treated chickens. The increase in T_4 at 2 h following injection of TSH was more pronounced in starved chickens (> 3-fold) than in fed birds (2-fold) (table III).

DISCUSSION AND CONCLUSION

These results confirm previous observations made both in adult layers (0.5–10 μg/kg) and adult broiler hens (1.5–24 μg/kg), *ie* that a physiological dose of TRH is not thyrotropic as judged by plasma concentrations of T_4 (Kühn *et al*, 1988, 1989), but a secretagogue for GH at every dose used. As a secondary effect, GH increases peripheral T_3 production. The results are not in contradiction with the earlier work of Klandorf *et al* (1978) who noted only a very small (1.3-fold) and short-lived increase in T_4 using an extremely high amount of TRH (± 75 μg/kg) in an adult layer strain, whereas a 2.3-fold increase in T_3 was observed which lasted for > 3 h.

However, the observed increase in plasma T_3 and simultaneous decrease in T_4 following TRH administration leaves open the possibility of TSH secretion and consequently T_4 release, which is immediately converted into T_3. This hypothesis can be ruled out, since a recent study has indicated that no change in T_4 occurs after TRH administration in adult dwarf hens who lack GH receptors and therefore are unable to

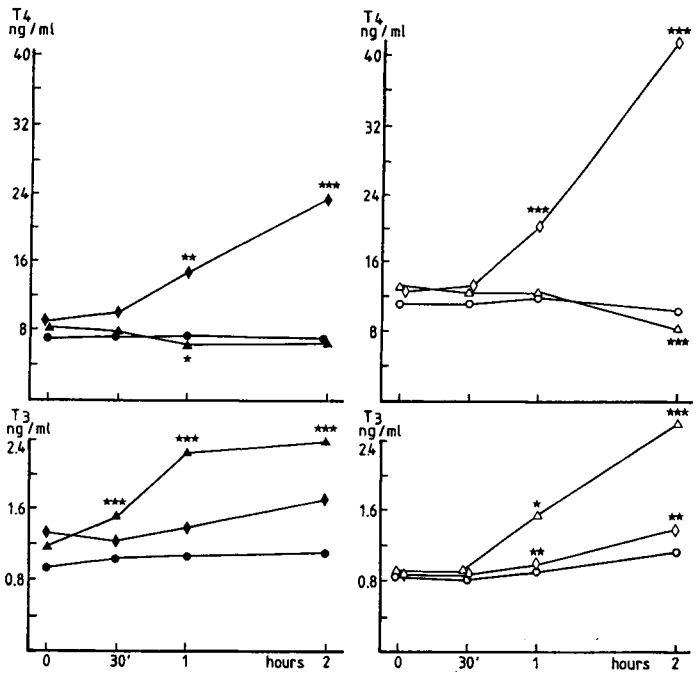

Fig 2. Influence of an injection at zero time of saline (● O), 100 µg bTSH (◆ ◊) or 100 µg oGH (▲ Δ) on plasma concentrations (ng) or T_3 and T_4 in normal fed (closed marks) or food deprived chickens for 7 d (open marks) * $P < 0.05$; $P < 0.01$; *** $P < 0.001$ paired *t*-test (exp 2).

show a GH mediated conversion of T_4 into T_3. On the other hand, in this experiment T_4 decreased in normal hens following TRH stimulation. Moreover, the stimulation of T_4 release by TSH is so dramatic in the present experiment that a much more pronounced increase in T_3 should be detectable.

These results are therefore in contrast with observations made in embryos (Kühn *et al*, 1988) or growing chickens (Michels *et al*, 1986) that TRH is thyrotropic and increases T_4 together with T_3. In dwarf embryos (Kühn *et al*, 1990) or dwarf chicks (Michels *et al*, 1986) who are unable to convert T_4 into T_3 due to a lack of GH receptors, plasma T_3 concentrations are not increased following the TRH challenge but the T_4 release is normal.

The observation that food deprivation lowers plasma concentrations of T_3 and increases T_4 (May, 1978; Decuypere and Kühn, 1984; Kühn *et al*, 1987) and GH (Harvey *et al*, 1988), has been confirmed in this study. It is also known that a hypothyroid state or food restriction (Lauterio and Scanes, 1988; Harvey *et al*, 1988) promotes TRH-induced GH secretion, which

Table III. Analysis of variance (with polynomials for repeated measurements). Influence of an injection of 100 µg TSH, 100 µg oGH or saline (= Treat) on the time dynamics of plasma concentrations of T_3 and T_4 in normal fed, and 3- and 7-d starved chickens (= Feed) (Exp 2).

	Main effects			*Interactions*			
	Feed (F)	*Treat (Tr)*	*Time (Ti)*	*F x Tr*	*F x Ti*	*Tr x Ti*	*F x Tr x Ti*
T_3 general	=0.016	<0.0001	<0.0001	NS	=0.009	<0.0001	NS
Zero time	=0.007	NS	–	NS	–	–	–
15 min	<0.0001	0.02	–	NS	–	–	
1 h	=0.009	<0.0001	–	NS	–	–	–
2 h	NS	<0.0001	–	NS	–	–	–
T_4 general	<0.0001	<0.0001	<0.0001	NS	=0.04	<0.0001	<0.0001
Zero time	<0.0001	NS	–	NS	–	–	–
15 min	<0.0001	=0.05	–	NS	–	–	–
1 h	<0.0001	<0.0001	–	NS	–	–	–
2 h	<0.0001	<0.0001	–	=0.002	–	–	–

is confirmed in our study, suggesting that GH release in the adult chicken is partly under tonic thyroidal inhibition. However, in the present study circulating levels of T_3 are not consistently decreased following food deprivation, which is in agreement with the observation that prolonged starvation (7 d) results in increased T_3, suggesting a reactivation of the peripheral monodeiodination after a certain period of starvation (Verheyen *et al,* 1983). Moreover, plasma T_4, which can inhibit GH release in the growing hen (Harvey, 1990) is increased in this study during food deprivation, but apparently unable to inhibit TRH-induced GH release.

It has been claimed that food deprivation can remove somatostatin inhibition on GH release and therefore promotes TRH-induced GH release (Lauterio and Scanes, 1988). However, no change in GH response was observed, following hGRF challenge in chicks following somatostatin neutralization (Buonomo *et al,* 1987). It has also been shown that somatostatin administration decreases T_4 release in the fowl (Lam *et al,* 1986; Iqbal *et al,* 1989). This at least would explain why in our study TSH administration provoked a much more pronounced increase in plasma concentration of T_4 in starved than in fed chickens.

Regardless, no thyrotropic effect of TRH on T_4 can be demonstrated; particularly after prolonged food deprivation, the GH response to TRH and TSH-induced T_4 release is dramatically increased.

However, the question remains as to why TRH is unable to stimulate the release of TSH in the adult chicken. It has been shown that in the chick more TRH receptors are present in the caudal lobe of the pituitary, which is primarily composed of somatotropes, than in the cephalic lobe, to which the thyrotropes are confined (Harvey and Baidwan, 1989). Apparently the TRH dose needed to stimulate the release of T_4 is at least 50 times greater than that which provokes GH release and peripheral T_3 production in the adult chicken (see

above). It is therefore possible that other hypothalamic factors may control the release of thyroid hormones from the thyroid, as has been demonstrated in amphibians (Denver, 1988; Jacobs *et al*, 1988) or in the chick embryo (Meeuwis *et al*, 1989).

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