Volume 310, number 1, 5–8 FEBS 11547 September 1992

Growth hormone acutely decreases type III iodothyronine deiodinase in chicken liver

V.M. Darras[a], L.R. Berghman[b], A. Vanderpooten[a] and E.R. Kühn[a]

[a]*Laboratory of Comparative Endocrinology, K.U. Leuven, Naamsestraat 61, B-3000 Leuven, Belgium* and [b]*Laboratory for Neuroendocrinology and Immunological Biotechnology, K.U. Leuven, Naamsestraat 59, B-3000 Leuven, Belgium*

Received 10 July 1992

Growth hormone (GH) increases plasma T_3 and decreases plasma T_4 in 18-day old chicken embryos, in newly hatched chicks and in adult chickens within 2 h after injection. The in vivo increase in T_3 can be linked to an increased in vitro T_3 recovery from liver homogenates incubated with T_4. Specific type I and type III deiodinase tests (5'D-I and 5D-III), however, show that GH has no effect at all on the amount of hepatic type I enzyme (catalyzing T_4 deiodination to T_3) but acutely decreases the amount of type III enzyme (catalyzing T_3 deiodination). This suggests that the GH-induced increase in plasma T_3 is not due to an increased T_3 production, but is the result of a decreased T_3 breakdown. The lack of a stimulatory effect of GH injection in 3-day-old fed chicks might be the combined result of a low hepatic type III enzyme level and a low GH receptor availability at that stage.

Iodothyronine deiodinase; Thyroid hormone; Growth hormone; chicken

1. INTRODUCTION

The stimulatory effect of growth hormone (GH) administration on circulating 3,3',5-triiodothyronine (T_3) levels has been demonstrated in a variety of vertebrates. In fish, it was shown in eels [1] and in rainbow trout [2]. In birds, T_3 rises were found in chickens [3,4] and quail (Kühn et al., unpublished results) and for mammals a stimulation was reported in man [5–8], in newborn lambs [9], in cattle [10] and in dwarf goats [11]. In most of these studies the in vivo effect of GH could be linked to an increased in vitro T_3 recovery from liver homogenates or microsomal fractions incubated with 3,3',5,5'-tetraiodothyronine (T_4), and it was concluded that GH stimulates the 5'-monodeiodination (5'D) of T_4 to T_3. In the rainbow trout and the eel, this indeed seems the most probable explanation, since under normal conditions only 5'D activity is found and T_3 itself is not a substrate for further deiodination [1,12]. In birds and mammals, however, both 5'D and 5-monodeiodination (5D) activity are present. This results in a far more complex degradation pattern. T_4 can be converted by 5'D to T_3 and by 5D to 3,3',5'-triiodothyronine (rT_3), while T_3 can be further deiodinated by 5D to 3,3'-diiodothyronine (T_2), and these processes all influence the final T_3 concentration measured in the in vitro test.

In mammals, different types of iodothyronine deiodinating enzymes (type I, II and III) have been characterized (review [13]), and related enzymes seem to be present in other vertebrates. It has been shown that in chicken liver both type I and type III activity are present and that the relative importance of both types of enzymes changes during late embryonic and early posthatch life [14–16]. During this same period, considerable changes have been found in the circulating GH levels and the total amount of hepatic GH receptors [15,17]. In view of the changing effects of a chicken GH (cGH) injection on peripheral monodeiodination activity during embryonic, early posthatch and adult life [18,19], it seemed interesting to investigate the acute effect of GH on the amount of type I and type III enzyme separately.

In the present study, hepatic type I (5'D-I) and type III (5D-III) activity have been measured following a single cGH injection in 18-day-old embryos (E18), newly hatched chicks (C0), chicks of 3 days old (C3) and adult chickens. In addition, plasma GH levels and hepatic GH receptor numbers have been measured to look for possible interactions with the changing effectiveness of the injected GH to increase plasma T_3.

2. MATERIALS AND METHODS

The fertilized eggs used in this study were purchased from Euribrid Belgium and belonged to a commercial layer strain (White Hisex). The eggs were incubated in a forced-draft laboratory incubator at 37.8°C, 30 mmHg vapour pressure, continuous lighting and were turned once every hour through angles of 45° (start of incubation = day 1). Posthatch chickens were kept in an acclimated room with a 14L/10D photo period and water and feed available ad libitum.

The cGH used for injections was purified by affinity chromatogra-

Correspondence address: V.M. Darras, Laboratory of Comparative Endocrinology, Naamsestraat 61, B-3000 Leuven, Belgium. Fax: (32) (16) 283902

phy [20]. All injections were given intravenously and consisted of 20 μg cGH/100 μl/animal or 100 μl 0.9% saline for controls. The animals were killed 2 h (E18-C3) or 1.5 h (adults) after injection. Blood samples were taken and the livers were immediately frozen in liquid nitrogen. For stages E18-C3, 10 individual livers from each group were used for preparation of homogenates (1 g/4 ml buffer) as described before [19]. From each group, 15–30 (depending on the age) other livers were collected, yielding 10 different pools/group for preparation of microsomal (Mx) fractions [15]. For adult chickens, 10 individual livers/group were used for preparation of Mx fractions.

Three different assays were used for measuring monodeiodinating activity:

1. T_4 to T_3 conversion analyzed by radioimmunoassay of T_3 after incubation of liver homogenates with T_4 [19]. The final incubation conditions used were 0.26 μM T_4, 2 mM DTT, 2 mM EDTA and ± 5 mg total protein/ml (60 min, 37°C).
2. 5'D-I assay based on the deiodination of ^{125}I-labeled rT_3, the preferred substrate, by liver Mx fractions [15]. The final incubation conditions used were 1 μM rT_3, 5 mM DTT, 2 mM EDTA and 100 μg Mx protein/ml (30 min, 37°C).
3. 5D-III assay based on the deiodination of ^{125}I-labeled T_3, the preferred substrate, by liver Mx fractions in the presence of excess rT_3 and 6-propyl-2-thiouracil (PTU) to block type I deiodinase activity [15]. The final incubation conditions used were 1 nM T_3 + 1 μM rT_3 + 1 mM PTU, 10 mM DTT, 2 mM EDTA and 100 μg Mx protein/ml (60 min, 37°C).

Plasma T_3, T_4 and GH levels were measured by radioimmunoassay as described before [15], while hepatic GH receptors were quantified following the method described by Vanderpooten et al. [17]. For stages E18–C3, the same animals were used for all measurements, while for the adult chickens, GH receptors were measured on a separate control group of the same stock of animals.

3. RESULTS

In embryonic and early posthatch chicks, cGH injection clearly increased plasma T_3 at stage E18 and C0, but not at stage C3, while it significantly decreased plasma T_4 at stages C0 and C3 (Table I). Control levels of GH increased from stage E18-C3, while the circulating levels 2 hours after injection were still increased at all stages (Table 1). The hepatic GH receptor level was high at stage E18, and low at stages C0 and C3, and GH injection decreased the number of GH receptors at all stages (Table I).

The control levels of hepatic 5'D-I activity showed only minor changes, while 5D-III activity decreased dramatically between stages E18–C3 (Fig. 1). The amount of T_3 recovered in the T_4 to T_3 conversion test was increased after GH injection at stages E18 and C0, but not at stage C3 (Fig. 2). GH induced no significant changes in 5'D-I activity at any stage, while it decreased 5D-III activity at stages E18 and C0, but not C3 (Fig. 1).

In adult chickens, GH injection increased plasma T_3, decreased plasma T_4 and hepatic 5D-III activity and had no influence on hepatic 5'D-I activity (Table II). Control levels were low for 5D-III activity and were about one half of E18–C3 levels for 5'D-I activity (Table II). The concentration of plasma GH and the specific hepatic GH binding were in the same range as for stage E18 (Table II).

4. DISCUSSION

The GH-induced changes in plasma T_3 and T_4 and the changes in the in vitro T_4 to T_3 conversion in liver homogenates from stages E18-C3 confirm earlier results in embryonic and posthatch chickens [3,4,18,19]. They all point in the direction of a stimulatory effect of GH on the 5'D of T_4 to T_3. The effect of GH treatment on the T_4 to T_3 conversion by liver homogenates suggests that it is mediated through a change in the amount of enzyme responsible for the T_4 to T_3 conversion, namely the type I enzyme. However, specific measurements of 5'D-I activity under saturating conditions, giving reliable estimates of V_{max} [15], clearly show that GH has no stimulatory effect at any stage on the amount of type I enzyme present in liver cells. 5D-III measurements, on the contrary, prove that GH significantly reduces the amount of hepatic type III deiodinase at all stages where plasma T_3 is increased (E18, C0 and adults). This indicates that the increase in plasma T_3 might not be due to an increased T_3 formation, but rather to a decreased T_3 breakdown as a result of a GH-induced inactivation/degradation of the type III deiodinase. This mechanism shows some similarities with the rapid changes in type II deiodinase in mammalian pituitary in response to hyper- or hypothyroidism [21], and is to our knowledge the first observation of an acute regulation of type III deiodinase.

The observed decrease in 5D-III activity is also in accordance with the decreased plasma rT_3 levels found after GH injection in previous experiments [18,19], since

Table I

Plasma T_3, T_4 and GH levels and hepatic GH receptor binding in embryonic and posthatch chicks 2 h after injection of saline(controls) or 20 μg cGH/animal.

	Controls	cGH
plasma T_3 (pmol/ml)		
E18	0.30 ± 0.05^{c}	$1.87 \pm 0.37^{***}$
C0	2.61 ± 0.13^{b}	$4.19 \pm 0.10^{***}$
C3	4.13 ± 0.18^{a}	4.04 ± 0.08
plasma T_4 (pmol/ml)		
E18	6.43 ± 1.15^{ab}	4.01 ± 0.64
C0	8.13 ± 0.88^{a}	$4.15 \pm 0.65^{***}$
C3	4.46 ± 0.50^{b}	$2.54 \pm 0.28^{**}$
plasma GH (ng/ml)		
E18	7.6 ± 0.5^{b}	$1116 \pm 128^{***}$
C0	35.7 ± 2.8^{b}	$707 \pm 55^{***}$
C3	72.5 ± 17.3^{a}	$241 \pm 14^{***}$
GH receptor binding (% B/mg prot)		
E18	40.5 ± 2.0^{a}	$6.93 \pm 0.51^{***}$
C0	4.10 ± 0.40^{b}	$1.89 \pm 0.40^{**}$
C3	4.29 ± 0.48^{b}	$1.95 \pm 0.87^{*}$

Values represent mean ± S.E.M. for 20 animals/group (6 pools/group for receptor binding).

^{a,b,c}Means of control groups with the same letter are not significantly different ($P<0.05$, ANOVA). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to control group of the same age (ANOVA).

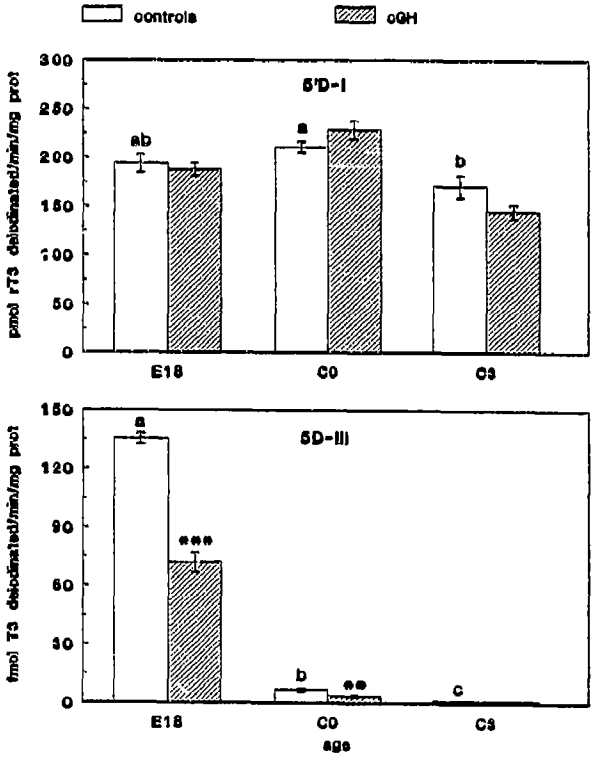

Fig. 1. 5'D-I and 5D-III activity in hepatic microsomal fractions of embryonic and posthatch chicks 2 h after injection of saline(controls) or 20 μg cGH/animal. Values represent mean ± S.E.M. for 10 different pools/group.

[a,b,c]Means of control groups with the same letter are not significantly different (ANOVA, $P<0.05$). $^{**}P<0.01$, $^{***}P<0.001$ compared to control group of the same age (ANOVA).

this enzyme is also responsible for at least part of the T_4 to rT_3 conversion. The decrease in plasma T_4 cannot be directly linked to the change in type III deiodinase level. It could however be due to a negative feedback on thyroidal T_4 release caused by the increased plasma T_3

Table II

Plasma T_3, T_4 and GH and hepatic 5'D-I activity, 5D-III activity and GH receptor binding in adult chickens 1.5 h after injection of saline(controls) or 20 μg cGH/animal

	Controls	cGH
plasma T_3 (pmol/ml)	1.28 ± 0.08	1.75 ± 0.22*
plasma T_4 (pmol/ml)	4.38 ± 0.46	3.04 ± 0.51*
5'D-I activity (pmol rT_3 deiodinated /min/mg prot)	81.7 ± 6.2	84.8 ± 8.7
5D-III activity (fmol T_3 deiodinated /min/mg prot)	1.16 ± 0.13	0.72 ± 0.08*
plasma GH (ng/ml)	14.8 ± 2.6	20.4 ± 2.8
GH receptor binding (% B/mg prot)	32.6 ± 2.3	

Values represent mean ± S.E.M. for 10 animals/group (6 pools/group for receptor binding). *$P<0.05$ compared to control group (ANOVA).

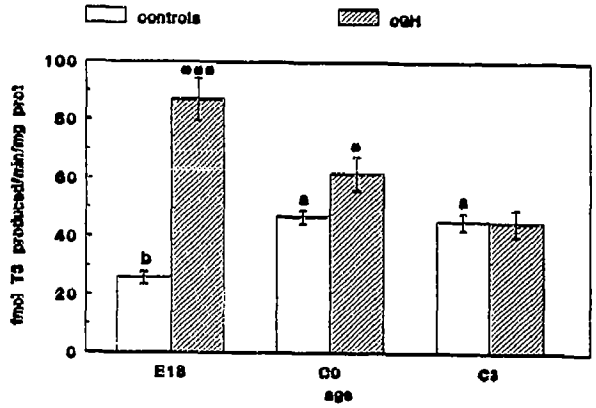

Fig. 2. T_4 to T_3 conversion in liver homogenates of embryonic and posthatch chicks 2 h after injection of saline(controls) or 20 μg cGH/animal. Values represent mean ± S.E.M. for 10 individual livers/group.

[a,b,c]Means of control groups with the same letter are not significantly different (ANOVA, $P<0.05$). *$P<0.05$, ***$P<0.001$ compared to control group of the same age (ANOVA)

levels. Another possibility is that GH can stimulate cellular uptake of T_4 in the chicken as has been shown in the rat [22], resulting in decreased plasma levels. The above results also clearly indicate that the T_4 to T_3 conversion test used before, cannot be regarded as a reliable estimate for pure 5'D activity. The amount of T_3 accumulating in incubations of T_4 with chicken liver homogenates is not only dependent on the rate of T_4 5'D to T_3 by the type I deiodinase but also on the activity of the type III enzyme which catalyzes the 5D of T_4 to rT_3 and of T_3 to T_2.

As was already seen in previous studies, the stimulatory effect of a GH injection on plasma T_3 is not present in normally fed C3 chicks, but is found again in adult chickens [4,18,19]. This corresponds with the observation that, although hepatic control levels of 5D-III activity are low in both groups, GH decreases 5D-III activity in adult but not in 3-day-old chickens. An explanation for this difference might be found in the number of receptor sites available for GH binding. C3 is the only stage in the above experiments where the liver shows both low 5D-III levels and a low amount of GH receptors. Those two factors taken together might result in the inability of the injected GH to increase plasma T_3 at that stage, even though its effect on plasma T_4 has not yet completely disappeared.

It can be concluded that the stimulatory effect of GH on plasma T_3 in the chicken is not mediated through an increase in the amount of hepatic type I deiodinase, but through an acute decrease in hepatic type III deiodinase, resulting in a decreased degradation of T_3. The combined effect of decreased hepatic GH receptor availability and decreased hepatic type III enzyme content, might explain the absence of T_3 stimulation in 3 days old chicks.

Acknowledgements: V. Darras and L. Berghman were supported by the Belgian National Fund for Scientific Research. This study was partially financed by a research grant from the Belgian National Fund for Scientific Research (F.K.F.O. 2.0039.90). We thank L. Noterdaeme, F. Voets, W. Van Ham and L. Grauwels for their valuable technical assistance.

REFERENCES

[1] deLuze, A. and LeLoup, J. (1984) Gen. Comp. Endocrinol. 56, 308–312.
[2] MacLatchy, D.L. and Eales, J.G. (1990) Gen. Comp. Endocrinol. 78, 164–172.
[3] Berghman, L., Darras, V.M., Huybrechts, L.M., Decuypere, E., Vandesande, F. and Kühn, E.R. (1989) Reprod. Nutr. Dev. 29, 197–202.
[4] Kühn, E.R., Verheyen, G., Chiasson, R.B., Huts, C., Huybrechts, L., Van den Steen, P. and Decuypere, E. (1987) Horm. Metab. Res. 19, 304–308.
[5] Gacs, G. and Banos, Cs. (1981) Acta Endocrinol. (Copenh.) 96, 475–479.
[6] Grunfeld, C., Sherman, B.M. and Cavalieri, R.R. (1988) J. Clin. Endocrinol. Metab. 67, 1111–1114.
[7] Jørgensen, J.O.L., Pedersen, S.A., Laurberg, P., Weeke, J., Skakkebaek, N.E. and Christiaensen, J.S. (1989) J. Clin. Endocrinol. Metab. 69, 1127–1132.
[8] Sato, T., Suzuki, Y., Taketani, T., Ishiguro, K., Masuyama, T., Takata, I., Sano, M., Kawashima, H., Koizumi, S. and Nakajima, H. (1977) J. Clin. Endocrinol. Metab. 45, 324–329.
[9] Kühn, E.R., Van Osselaer, P., Siau, O., Decuypere, E. and Moreels, A. (1986) J. Endocr. 109, 215–219.
[10] Rumsey, T.S., Kahl, S., Norton, S.A., Eisemann, J., Elsasser, T.H., Hammond, A.C., Tyrrell, H.F. and Bitman, J. (1990) Dom. Animal Endocrinol. 7, 125–134.
[11] Iqbal, A., Cheema, A.M. and Kühn, E.R. (1990) Horm. Metab. Res. 22, 566–568.
[12] Shields, C.A. and Eales, J.G. (1986) Gen. Comp. Endocrinol. 63, 334–343.
[13] Leonard, J.L. and Visser, T.J., in: Thyroid Hormone Metabolism, (G. Hennemann Ed.), Dekker, New York, 1986, pp. 189–229.
[14] Borges, M., LaBourene, J. and Ingbar, S.H. (1980) Endocrinology 107, 1751–1761.
[15] Darras, V.M., Visser, T.J., Berghman, L.R. and Kühn, E.R. (1992) Comp. Biochem. Physiol. A, in press.
[16] Galton, V.A. and Hiebert, A. (1987) Endocrinology 120, 2604–2610.
[17] Vanderpooten, A., Huybrechts, L.M., Decuypere, E. and Kühn, E.R. (1991) Reprod. Nutr. Dev. 31, 47–55.
[18] Darras, V.M., Huybrechts, L.M., Berghman, L., Kühn, E.R. and Decuypere, E. (1990) Gen. Comp. Endocrinol. 77, 212–220.
[19] Darras, V.M., Vanderpooten, A., Huybrechts, L.M., Berghman, L.R., Dewil, E., Decuypere, E. and Kühn, E.R. (1991) Horm. Metab. Res. 23, 469–472.
[20] Berghman, L.R., Van Beeumen, J., Decuypere, E., Kühn, E.R. and Vandesande, F. (1988) J. Endocrinol. 118, 381–387.
[21] Leonard, J.L., Silva, J.E., Kaplan, M.M., Mellen, S.A., Visser, T.J. and Larsen, P.R. (1984) Endocrinology 114, 998–1004.
[22] Geelhoed-Duijvestein, P.H.L.M. (1990). Effects of growth hormone on the thyroid and thyroid hormone production. Ph. D. Thesis (Pasmans Offsetdrukkerij, The Hague).