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Thyroid hormone metabolism in poultry

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Thyroid hormone (TH) receptors preferentially bind 3,5,3'-triiodothyronine (T_3). Therefore the metabolism of thyroxine (T_4) secreted by the thyroid gland in peripheral tissues, resulting in the production and degradation of receptor-active T_3, plays a major role in thyroid function. The most important metabolic pathway for THs is deiodination. Another important pathway is sulfation, which is a reversible pathway that has been shown to interact with TH deiodination efficiency. The enzymes catalysing TH deiodination consist of three types. Type I deiodinase (D1) catalyses both outer ring (ORD) and inner ring deiodination (IRD). Type II deiodinase (D2) only catalyses ORD while type III (D3) only catalyses IRD. The three chicken deiodinase cDNAs have been cloned recently. These enzymes all belong to the family of selenoproteins. Ontogenetic studies show that the availability of deiodinases is regulated in a tissue specific and developmental stage dependent way. Characteristic for the chicken is the presence of very high levels of T_3 inactivating D3 enzyme in the embryonic liver. Hepatic D3 is subject to acute regulation in a number of situations. Both growth hormone and glucocorticoid injection rapidly decrease hepatic D3 levels, hereby increasing plasma T_3 without affecting hepatic D1 levels. The inhibition of D3 seems to be regulated mainly at the level of D3 gene transcription. The effect of growth hormone on D3 expression persists throughout life, while glucocorticoids start to inhibit hepatic D1 expression in posthatch chickens. Food restriction in growing chickens increases hepatic D3 levels. This contributes to the decrease in plasma T_3 necessary to reduce energy loss. Refeeding restores hepatic D3 and plasma T_3 to control levels within a few hours. It can be concluded that the tissue and time dependent regulation of the balance between TH activating and inactivating enzymes plays an essential role in the control of local T_3 availability and hence in TH activity.
Keywords. Thyroid hormones (metabolism of), deiodination, sulfation, poultry, chicken.

Métabolisme des hormones thyroïdiennes chez la volaille. Les récepteurs des hormones thyroïdiennes (HTs) lient par préférence la 3,5,3'-triiodothyronine (T_3). Par conséquent le métabolisme de la thyroxine (T_4), sécrétée par la glande thyroïde, dans les tissus périphériques menant à une production et à une dégradation de la T_3 active aux récepteurs joue un rôle majeur dans la fonction thyroïdienne. La voie métabolique principale des HTs est la désiodation. Une autre voie importante est la sulfatation, un processus réversible qui affecte l'efficacité de la désiodation des HTs. On distingue trois types d'enzymes qui catalysent la désiodation des HTs. La désiodase de type I (D1) affecte le noyau aromatique extérieur (ORD) et le noyau aromatique intérieur (IRD) de la T_4. La désiodase de type II (D2) catalyse exclusivement l'ORD, tandis que la désiodase de type III (D3) ne catalyse que l'IRD. Les cADNs des trois désiodases du poulet ont été clonés récemment. Ces enzymes appartiennent à la famille des sélénoprotéines. Des études ontogéniques indiquent que la régulation de la disponibilité des désiodases dépend du type de tissu et du stade de développement. La présence d'une forte teneur en D3 (qui inactive la T_3) dans le foie embryonnaire est caractéristique du poulet. La D3 hépatique est soumise à une régulation prononcée dans de nombreuses situations. L'injection d'hormone de croissance ainsi que l'injection de glucocorticoïdes diminuent rapidement la teneur hépatique en D3, ayant comme résultat une augmentation en T_3 plasmatique sans avoir affecté la teneur hépatique en D1. L'inhibition de la D3 paraît s'effectuer essentiellement au niveau de la transcription du gène de cette enzyme. L'effet de l'hormone de croissance sur l'expression de la D3 persiste pendant toute la vie, tandis que les glucocorticoïdes déclenchent une inhibition de l'expression hépatique de la D1 après l'éclosion des poussins. Dans des poulets en croissance, le jeûne augmente la teneur hépatique en D3. Ceci contribue à la diminution de la T_3 plasmatique, nécessaire afin de réduire la perte d'énergie. Une réalimentation rétablit le niveau hépatique en D3 et en T_3 plasmatique aux valeurs de contrôle en quelques heures. On peut conclure que la régulation tissulaire et temporelle de l'équilibre entre les enzymes qui activent ou inactivent les HTs, joue un rôle essentiel dans la régulation de la disponibilité locale de la T_3 et donc de l'activité des HTs.
Mots-clés. Hormones thyroïdiennes (métabolisme des), désiodation, sulfatation, volaille, poulet.

1. INTRODUCTION

Thyroid hormones (THs) have multiple effects on vertebrate metabolism and development. In homeothermic animals, THs regulate basal metabolic rate and are essential for the maintenance of high and constant body temperature. The effect of THs on protein and lipid metabolism is of a biphasic nature: in low physiological concentrations they are anabolic while at higher concentrations they are catabolic. During development THs stimulate both growth and differentiation (or maturation). Their action can be direct, indirect or permissive. Most of the actions of THs seem to be dependent on the binding to a nuclear thyroid hormone receptor (TR). Two major isoforms of these receptors are known (TRα and TRβ) and both of them preferentially bind 3,5,3'-triiodothyronine (T_3). The main secretory product of the thyroid gland, 3,5,3',5'-tetraiodothyronine or thyroxine (T_4), is considered to be a relatively inactive prohormone due to its low binding affinity to TRs. Consequently, the peripheral metabolism of T_4 by activating and inactivating pathways is very important in the regulation of the availability of receptor-active T_3 and hence of thyroid activity.

The most important metabolic pathway for THs is (mono)deiodination where one iodine is removed from the outer ring (outer ring deiodination or ORD) or from the inner ring (inner ring deiodination or IRD) of a iodothyronine molecule. Deiodination is an irreversible process which can lead to activation as well as inactivation of THs. Outer ring deiodination of T_4 is the only way to produce active T_3 and therefore ORD is important as an activating pathway. Inner ring deiodination of T_4 or T_3 can only lead to inactive iodothyronines, namely reverse T_3 (rT_3), respectively 3,3'-diiodothyronine (T_2), and therefore IRD is exclusively an inactivating pathway (**Figure 1**).

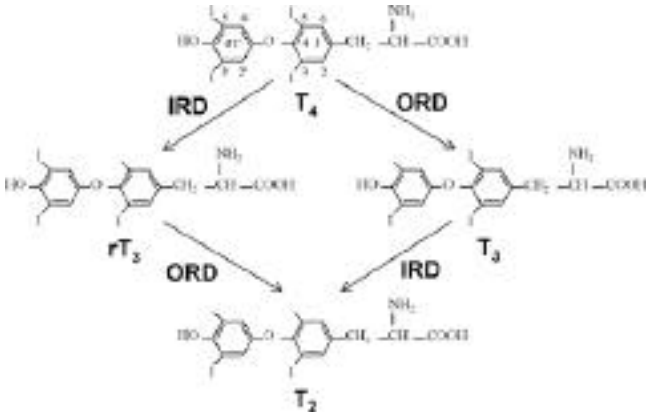

Figure 1. Stepwise deiodination of T_4 by outer ring deiodination (ORD) and inner ring deiodination (IRD) — *Désiodation progressive de la T_4 par des interventions sur le noyau aromatique extérieur (ORD) et sur le noyau aromatique intérieur (IRD).*

Deamination and decarboxylation of the alanine side chain as well as cleavage of the ether bond between the two iodothyronine rings are also irreversible pathways of TH metabolism. They always lead to loss of hormonal activity. Conjugation of the phenolic hydroxyl group with sulfate or glucuronic acid are reversible pathways of TH metabolism. Recently sulfation has been shown to interfere with TH deiodination (review by Visser, 1990).

2. THYROID HORMONE DEIODINATION

2.1. Characterisation of deiodinases

The enzymes catalysing deiodination of thyroid hormones have first been described in mammals, more specifically in rats. So far, three types have been identified: iodothyronine deiodinase type I (D1), type II (D2) and type III (D3). Type I is a multifunctional enzyme that catalyses both ORD and IRD. Its preferred substrate is rT_3 (above T_4 and T_3) with a K_m value in the low micromolar range. Type II only catalyses ORD with a substrate preference for T_4 over rT_3 (K_m in the low nanomolar range). Type III is a pure IRD enzyme with a substrate preference for T_3 over T_4 (K_m in the low nanomolar range) (reviews by Leonard, Visser, 1986; St. Germain, Galton, 1997). As a consequence only D1 and D2 can activate T_4 to T_3 while D3 has always an inactivating role. All three enzymes need reduced thiol groups as a cofactor and their activity can be blocked by inhibitors such as iodoacetate, iopanoate and aurothioglucose. Type I deiodinase can also be blocked by 6-n-propyl-2-thiouracil (PTU), while D2 and D3 are insensitive to this inhibitor. More recent studies have shown that the deiodinases in birds (and in other vertebrates as well) closely resemble the ones in mammals, although some fish species seem to possess a PTU-resistant D1 (Mol *et al.*, 1993; Sanders *et al.*, 1997).

So far, the only avian deiodinase cDNAs available are from chicken. Chicken D1 and D3 have been cloned a few years ago (Cogburn *et al.*, 1997; Van der Geyten *et al.*, 1997) while the sequence of chicken D2 cDNAwas only published a few months ago (Gereben *et al.*, 1999). The cDNAs for chicken D1 and D3 are approximately 1.4 kilobases long. Type I deiodinase is a 27 kDa protein (246 amino acids long). The exact length of the chicken D3 protein remains unknown since the D3 clone misses approximately 46 N-terminal nucleotides. Based on sequence homology with other D3 cDNAs, the estimated length of the chicken protein is 273 amino acids. The D2 cDNA is over 6 kilobases long and codes for a 31 kDa protein (279 amino acids long). The three chicken deiodinases, as all other deiodinases cloned so far, are selenoproteins having a selenocysteine in their catalytic site.

Selenocysteine is encoded by UGA which normally functions as a stop codon. Its incorporation in the protein is made possible by the presence of a stemloop structure in the 3' untranslated region of the mRNA, called selenocysteine insertion sequence (SECIS) element. Site directed mutation studies with rat D1 have shown that substitution of selenocysteine by cysteine enhances the efficiency of translation, but strongly reduces catalytic activity of the protein, showing that selenocysteine is indeed an important element in the enzyme's active centre (Berry *et al.*, 1992).

2.2. Tissue distribution of deiodinases

Ontogenetic studies of the distribution of deiodinases in embryonic chickens during the last week of incubation (total length 21 days) show that the different enzyme types are clearly expressed in a tissue specific way (Darras *et al.*, 1992b, 1999a; Van den Eynde *et al.*, 1999). The level of active enzyme has been measured for each enzyme type separately by specific *in vitro* tests under saturating conditions (estimate of V_{max}). The presence of the respective mRNAs has been investigated by Northern blot using ^{32}Plabelled probes. **Table 1** gives a summary of the activity results.

Type II activity is mainly restricted to the brain, although very low levels of activity are also found in lung tissue. Interestingly, the D2 activity in lung shows a peak at the moment of internal pipping, when the membrane to the air chamber of the egg is perforated and lung respiration starts. Type I activity is abundant in liver, kidney and intestine, medium in several other tissues and absent in muscle, skin and brain. The D1 activity increases in liver and intestine towards hatching but decreases in gonads and bursa of Fabricius. Type III activity is present in all embryonic tissues studied. By far the greatest D3 activity is found in embryonic liver where D3 levels around day 17 of incubation can be more than 2000 fmol T_3 deiodinated per mg protein and per minute compared to less than 20 in other tissues. In most tissues the mRNA expression pattern closely follows the profile found in activity levels. A detailed study of deiodinase expression in tissues of growing or adult chickens has not yet been published, but although the D3 activity levels tend to be lower in posthatch compared to embryonic animals, results from a number of separate studies strongly suggest the distribution of the enzyme types resembles the one in embryos. One exception may be D2 that is not found in embryonic liver while D2 mRNA is clearly expressed in adult chickens (Gereben *et al.*, 1999) as well as in newly hatched chicks (Van der Geyten *et al.*, unpublished results).

Probably the best illustration of the impact of ontogenetic changes in expression of activating and inactivating deiodinases on T_3 availability is the profile found in the embryonic chicken liver. The liver is a TH exporting tissue with high deiodinating activity and contributes to a high degree in the circulating levels of T_3. **Figure 2** shows the profile of plasma T_4 and T_3 and of hepatic D1 and D3 activity during the last week of embryonic development. Comparison of the profiles shows that although the levels of plasma T_4 and hepatic D1 slowly increase, T_3 levels stay low until the period shortly before internal pipping. This can be explained by the fact that hepatic D3 levels are very high till around day 18. When D3 expression decreases, T_3 degradation is strongly reduced, allowing for the accumulation of T_3 in the circulation (Darras *et al.*, 1992a). More recently the hepatic D1 and D3 activity profiles have been confirmed and shown to be parallel to D1 and D3 mRNA expression patterns (Van der Geyten *et al.*, 1997).

Table 1. Distribution of D1, D2 and D3 activity in tissues from embryonic chickens taken during the last week of embryonic development. +, ++, +++ indicate increasing levels of activity; - indicates absence of activity — *Aperçu des activités des désiodases D1, D2 et D3 dans divers tissus d'embryon de poulet durant la dernière semaine du développement embryonnaire. +, ++ et +++ réfèrent à des niveaux croissants d'activité ; - signifie l'absence d'activité.*

Tissue	D1	D2	D3
Liver	+++	-(?)	+++
Kidney	+++	-	++
Lung	++	+	+
Heart	+	-	+
Intestine	+++	-	+
Gonads	++	-	+++
Spleen	+	-	+
Bursa Fabricii	+	-	++
Skeletal muscle	-	-	+
Skin	-	-	+
Brain	-	+++	++
Pituitary	+	-	+

3. CONTROL OF DEIODINASE ACTIVITY

3.1. Different levels of control

In the study of the regulation of peripheral deiodination, it is important to keep in mind that regulation of *in vivo* enzyme activity is dependent on three main factors: enzyme availability, substrate availability and cofactor availability. Different techniques can be used to measure the level of the different deiodinases, including Northern blot and RT-PCR for mRNA quantification, Western blot and ELISA for protein measurement and kinetic radiometric tests for

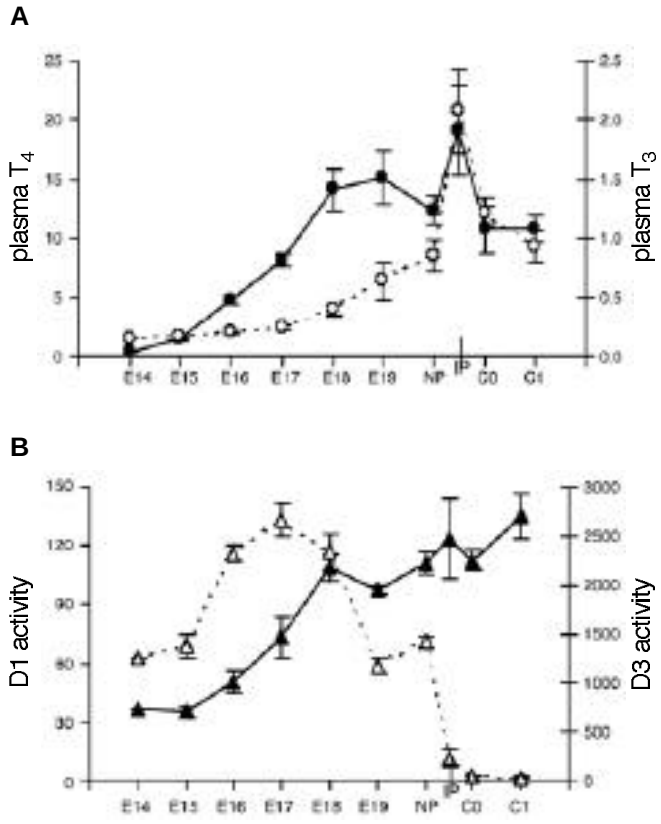

Figure 2. Ontogenetic profiles of (**A**) plasma T_4 (pmol/ml, ·¾·) and T_3 (pmol/ml, o– – –o) and (**B**) hepatic D1 activity (pmol rT_3 deiodinated/mg protein.min, ▲¾▲) and D3 activity (fmol T_3 deiodinated/mg protein.min, △– – – △) from 14-day-old embryos to 1-day-old chicks. Values represent mean ± SEM for groups of 10–20 animals — *Profils ontogéniques des teneurs plasmatiques (***A***) en T_4 (pmol/ml, ·¾·) et en T_3 (pmol/ml,* o– – –o*) et des activités hépatiques (***B***) en D1 [pmol de rT_3 désiodée/mg de protéine.min, ▲¾▲] et en D3 [fmol de T_3 désiodée/mg protéine.min, △– – –△] mesurées sur des embryons de poulet âgés de 14 jours jusqu'à l'âge de un jour après éclosion des poussins. Les valeurs représentent des moyennes ± écarts-types de groupes de 10 à 20 individus.*

estimation of the amount of active enzyme. Assessment of substrate availability is more difficult, since this depends on the activity of TH transporters which carry the hormone through the membrane in and out the cell. A number of putative TH transporters have recently been cloned in rat and human, but their affinity for TH is relatively low and the real *in vivo* TH transporter may still remain unknown. In birds no transporters have been identified yet and the possibilities to investigate intracellular TH availability are mainly limited to extraction of TH from the tissues followed by RIAmeasurement (Prati *et al.*, 1992) or *in vitro* studies on perfused whole organs or isolated cells (Docter, Krenning, 1990). Cofactor availability is even more difficult to estimate, since the *in vivo* cofactor for deiodinases has not yet been identified. It is generally accepted that reduced thiol groups are important and therefore dithiotreitol (DTT) is widely used as cofactor for *in vitro* assays.

The following sections on hormonal and nutritional regulation of deiodination will mainly focus on changes in enzyme activity and expression.

3.2. Control by hormones

Thyroid hormones themselves are clearly affecting deiodinase expression. Molecular studies in mammals and amphibians have proven that both D1 and D3 gene transcription are upregulated by T_3 (Berry *et al.*, 1990; St. Germain *et al.*, 1994). In poultry thyroid hormone dependent changes in deiodinase activity have been studied in the brain of growing chickens. Two days after thyroidectomy serum levels of T_3 decreased to 3% of the levels in sham operated animals. In brain tissue, however, T_3 levels were maintained at approximately 90% of control levels (Rudas *et al.*, 1993). Measurement of *in vitro* enzyme activity showed that D2 activity in the brain was increased about ninefold while D3 activity was reduced by a factor three (Rudas *et al.*, 1993) showing that local changes in T_3 producing and degrading activities contribute to brain T_3 homeostasis.

Early studies from our own group with ovine prolactin (oPRL) and ovine growth hormone (oGH) showed that a single injection of oGH increased plasma T_3 in embryonic as well as adult chickens, while oPRL had the same effect but only in embryonic chickens. At the same time, these hormones increased the recovery of T_3 when liver homogenates were incubated with T_4, suggesting changes in hepatic deiodination were the reason behind the increase in plasma T_3 (Decuypere, Kühn, 1985; Kühn *et al.*, 1986, 1987). More recent studies using homologous immunoaffinity purified chicken growth hormone (cGH) (Berghman *et al.*, 1988) and recombinant chicken prolactin (cPRL) (Ohkubo *et al.*, 1993) as well as type-specific deiodinase activity tests confirmed the stimulatory effect of cGH but not of cPRLon plasma T_3 in 18-day-old embryos. Two hours after intravenous injection cGH clearly increases plasma T_3 and decreases plasma T_4. At the same time it drastically inhibits *in vitro* hepatic D3 activity while having no effect on hepatic D1 (Darras *et al.*, 1992a). This again suggests that degradation of T_3 by D3 is an important factor in the regulation of plasma T_3 levels. Although the inhibitory effect of cGH on D3 is most clearly seen in the liver, where control levels are high, similar effects are found on D3 in kidney and hypothalamus (**Table 2**). Injection of cPRLdecreases plasma T_4 but has no effect on plasma T_3. Hepatic D1 does not change while hepatic D3 is very shortly increased (Kühn

Table 2. Changes in D3 activity (fmol T_3 deiodinated/mg protein.min) 2 hours after a single injection of 2.5 µg cGH in 18-day-old chicken embryos — *Variation de l'activité D3 [exprimée en fmol de T3 désiodée/mg de protéine.min] consécutive à l'injection de 2,5 µg d'hormone de croissance de poulet (cGH) à des embryons de poulet âgés de 18 jours. Les mesures renseignées sont effectuées deux heures après injection de tampon (*saline*) ou d'hormone (*cGH*).*

	Saline	cGH
Liver	1085 ± 99[a]	482 ± 67 ***
Kidney	0.94 ± 0.13	0.59 ± 0.06 *
Hypothalamus	0.97 ± 0.10	0.59 ± 0.05 **
Skin	2.57 ± 0.27	0.93 ± 0.07 ***

[a] Values represent mean ± SEM per group of 12 liver samples, 6 hypothalamus pools and 4 kidney or skin pools
*, **, ***: p < 0.05, < 0.01, < 0.001 with Student's T-test.

et al., 1996). The divergent results obtained earlier with oPRL can be explained by the finding that oPRL(as well as oGH) is able to bind to the chicken GH receptor while recombinant cPRL is not (Kühn *et al.*, 1996).

Some studies with single cGH injections suggested that GH could stimulate plasma T_3 in embryonic, newly hatched and adult chickens, but not during the period of rapid growth (Darras *et al.*, 1990). Later experiments showed that the relative insensitivity of growing chickens to cGH injection is due to the high endogenous levels of GH in circulation, linked to low hepatic GH receptor availability and low hepatic D3 levels. Injection of antiserum against cGH in growing chickens clearly decreases plasma T_3, while cGH injection in hypophysectomised young chickens with very low levels of circulating GH and T_3 can increase plasma T_3 (Darras *et al.*, 1993). Recently it was also shown that repeated pulsatile administration of recombinant cGH in growing broiler chickens also increases plasma T_3 while inhibiting hepatic D3 expression and that the catabolic effect of increased T_3 on protein synthesis may contribute to the lack of growth promoting activity of GH administration in broiler chickens (Vasilatos-Younken *et al.*, 1998).

In chickens a close interaction is present between the thyroidal and adrenal axis. Corticotropin releasing factor (CRF) is known to stimulate thyrotropin secretion and hence thyroidal T_4 secretion, while corticosterone (B) exhibits a negative feedback on T_4 secretion (Geris *et al.*, 1996, 1999). At the peripheral level B has profound effects on both D1 and D3 activity which are different according to the developmental stage of the animal. In embryonic chickens a single injection of B or of its long acting synthetic analogue dexamethasone (DEX) decreases plasma T_4 but strongly increases plasma T_3 (Decuypere *et al.*, 1983; Darras *et al.*, 1996). This is accompanied in the liver by a rapid inhibition (within 2 h) of the high D3 levels by B or DEX while the long acting DEX also increases D1 at 24 and 48 h after injection (Darras *et al.*, 1996). In 8-day-old posthatch chicks B and DEX decrease plasma T_4 but to a lesser extent also plasma T_3. Their inhibitory effect on the much lower hepatic D3 levels is reduced or even absent, but they now have a clear inhibitory effect on hepatic D1 (**Figure 3**).

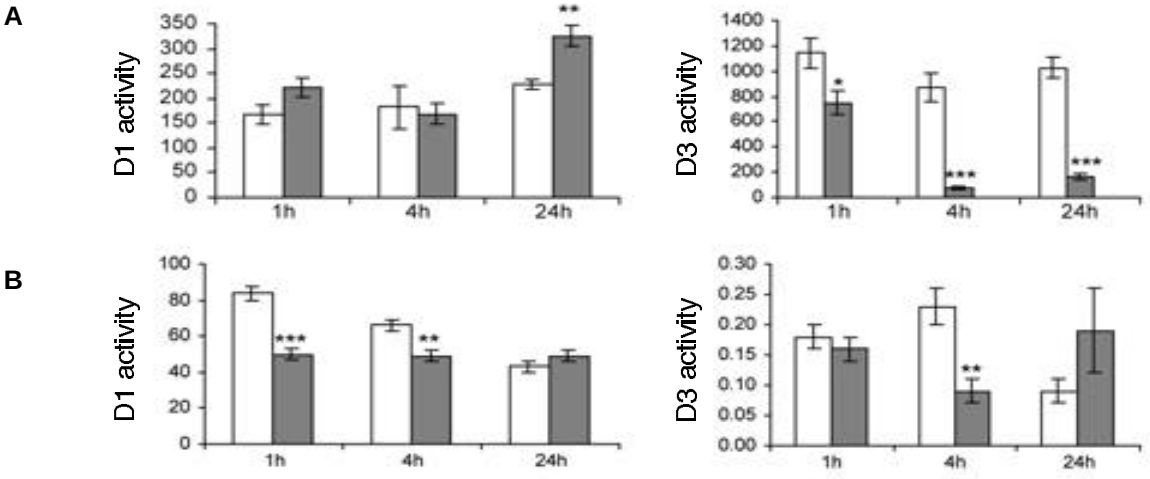

Figure 3. Effect of a single injection of saline (□) or 10–20 µg DEX (■) on hepatic D1 activity (pmol rT_3 deiodinated/mg protein.min) and D3 activity (fmol T_3 deiodinated/mg protein.min) in 18-day-old embryos (**A**) and in 8-day-old chicks (**B**). Values represent mean ± SEM for groups of 5 pools of 2 embryos or of 10 individual chicks. *, **, ***: p < .05, .01, .001 by Student's T-test — *Effet sur l'activité hépatique en D1 [pmol de rT_3 désiodée/mg protéine.min] et D3 [fmol de T_3 désiodée/mg protéine.min] d'une injection unique de tampon (□) ou de 10–20 µg de dexaméthasone (DEX, ■) à des embryons de poulet âgés de 18 jours (**A**) et à des poussins de 8 jours (**B**). Les valeurs représentent des moyennes ± écarts-types de groupes d'embryons en 5 pools de 2 individus ou de 10 poussins. *, **, ***: p < ,05, ,01, ,001 dans le test t de Student.*

Recently we have studied in more detail the acute inhibition of hepatic D3 by cGH and DEX in chicken embryos to determine at what level regulation takes place. Quantitative measurement of D3 mRNA expression by competitive RT-PCR shows that both cGH and DEX decrease mRNA levels to reach a minimum within 30 min after injection, proving the involvement of pretranslational regulation (Van der Geyten *et al.*, 1999a). Additional studies including actinomycine D and cycloheximide to block transcription or translation suggest that both D3 mRNA half-life and D3 enzyme half-life are not changed by cGH or DEX (Darras *et al.*, 1999b; Van der Geyten *et al.*, 1999c) what leads to the hypothesis that the regulation takes place exclusively at the level of gene transcription. This hypothesis is presently tested with additional experiments.

3.3. Control by nutrition

In chickens, as in all vertebrates studied so far, food restriction decreases plasma T_3. Unlike in many other vertebrates, food restriction in chickens increases circulating levels of T_4 (May, 1978; Klandorf, Harvey, 1985). The effects of partial food restriction as well as of a two day fasting period on deiodinase levels have been studied in young broiler chickens (Darras *et al.*, 1995; Van der Geyten *et al.*, 1999b). The most prominent change is an increase in hepatic D3 levels, while D3 in kidney and brain tissue decreases. Brain D2 activity levels tend to increase, while hepatic and renal D1 remain unaffected. The increase in hepatic D3 activity is paralleled by increased D3 mRNA levels, suggesting that D3 gene expression is indeed upregulated (Van der Geyten *et al.*, 1999b). Since the liver is the organ which contributes most to plasma T_3 levels, increase of hepatic D3 expression could be a way to reduce T_3 supply to tissues over the whole body and hence contribute to a decrease of energy expenditure. In some important organs such as brain and kidney, decrease of D3 and increase of D2 expression could help to keep local T_3 levels above a critical limit despite the lower T_3 supply. At the moment of refeeding, hepatic D3 levels very quickly return to basal levels, followed by an increase and normalisation of plasma T_3 within a few hours after refeeding (Darras *et al.*, 1997; Buyse *et al.*, 2000).

Nutritional effects on deiodination are not restricted to the amount of food available. Changes in food composition may also affect TH deiodination. Increasing the percentage of fat in isocaloric food alters TH economy in the same way as food restriction does (Bartha, 1993).

4. THYROID HORMONE SULFATION

Sulfation of THs is catalysed by sulfotransferases, while desulfation is catalysed by arylsulfatases. In mammals, especially rat and human, a whole family of sulfotransferases and of arylsulfatases have been identified (reviewed by Coughtrie *et al.*, 1998; Visser *et al.*, 1998). The exact nature of these enzymes in birds remains to be determined. A recent study of sulfation activity in a number of chicken tissues shows the presence of significant amounts of sulfotransferase activity in liver, kidney and brain cytosol. As in rats, T_2 is by far the preferred substrate for sulfation, followed by T_3 and rT_3 and finally T_4 (Reyns *et al.*, 1999). Sulfation is already present in embryonic tissues and the activity profiles during the last week of embryonic development demonstrate that, as for deiodination, sulfation is regulated in a tissue-specific way (Reyns *et al.*, 1999). Despite the presence of sulfotransferase activity in several tissues, circulating levels of T_3 sulfate and T_4 sulfate in chicken embryos and early posthatch chicks are very low (Darras *et al.*, unpublished results).

In the body sulfation generally plays a role as a detoxification process. It increases the water solubility of hydrophilic substances, such as TH, thereby facilitating their urinary and biliary excretion. For TH, sulfation also allows for the transport and temporary storage of receptor-inactive T_3 sulfate which can be reactivated in the presence of sulfatases. The role of sulfation in TH deiodination has only been recognised recently by studies in rat. Sulfated iodothyronines are no substrates for deiodination by D2 and D3. On the other hand, sulfation strongly facilitates IRD of T_4 (by a factor 200) while it completely blocks ORD of T_4. Inner ring deiodination of T_3 sulfate by D1 is facilitated in a similar way (by a factor 40) while ORD of rT_3 remains unaffected after sulfation (Visser *et al.*, 1990). In view of the strong homology between rat and chicken D1, it can be hypothesised that sulfation affects TH deiodination by D1 in a similar way in chickens. However, in view of the low levels of iodothyronine sulfates detected so far, it remains to be elucidated to what degree sulfation contributes to *in vivo* TH deiodination in chickens.

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