Developmentally defined regulation of thyroid hormone metabolism by glucocorticoids in the rat

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Abstract

Glucocorticoids are known regulators of thyroid function in vertebrates. In birds they have clear tissue-specific and age-dependent effects on thyroid hormone metabolism. In mammals, however, few studies exist addressing these aspects using an *in vivo* model system. We therefore set out to examine the acute effects of a single dose of dexamethasone (DEX) on plasma 3,5,3'-tri-iodothyronine (T_3) and thyroxine (T_4) levels, as well as on the activity of the different deiodinases in liver, kidney and brain in the developing rat. In 20-day-old fetuses (E20), glucocorticoids had no effects on circulating thyroid hormone levels despite their clear effects on hepatic and renal deiodinases, thereby indicating that under these conditions circulating thyroid hormone levels are more dependent on thyroidal secretion than on peripheral deiodination. In contrast, in 5-day-old rat pups, DEX did not seem to have any effects on hepatic and renal T_3 production (via the type I

deiodinase), whereas type III deiodinase (D3) activity in both these tissues increased significantly. These observations therefore suggested that the DEX-induced increase in circulating T_3 levels is a direct consequence of the increase in plasma T_4 levels. In 12-day-old pups (P12), however, the main effect of glucocorticoids on circulating T_3 levels was by increasing inner ring deiodination through induction of D3 in both liver and kidney. Finally, in the brain, glucocorticoids stimulated thyroid hormone activity only during a short period of time (between E20 and P12) that largely overlaps with the transient window in time during which brain development is thyroid hormone sensitive. This was in contrast to the E20 and P12 brain, where the glucocorticoid-induced changes in type II deiodinase and D3 seemed to favor a *status quo* in local T_3 availability.

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Introduction

The thyroid secretes predominantly 3,5,3',5'-tetra-iodothyronine (thyroxine, T_4) that has to be converted into 3,5,3'-tri-iodothyronine (T_3) in order to bind to a nuclear receptor and exert its full biological activity. This conversion occurs in a wide array of tissues and is catalyzed by a group of selenocysteine-containing oxidoreductases called iodothyronine deiodinases. So far, three types of deiodinases have been identified. The type I deiodinase (D1), which is capable of both activation (outer ring deiodination, ORD) and inactivation (inner ring deiodination, IRD), is a high K_m ($\approx 0.2 \times 10^{-6}$ M for reverse T_3 (rT_3)), high V_{max} enzyme which is highly sensitive to inhibition by 6-*n*-propylthiouracyl (PTU). The type II deiodinase (D2), on the other hand, is a low K_m ($\approx 2 \times 10^{-9}$ M for T_4), low V_{max} enzyme that only has ORD activity and is not inhibited by PTU. Like D2, the type III deiodinase (D3) is also a selective low K_m ($\approx 7 \times 10^{-9}$ M for T_3), low V_{max} enzyme that is only capable of IRD and is relatively insensitive to PTU (Bianco *et al.* 2002).

Glucocorticoids have been demonstrated to affect thyroid function by interacting at both the central and peripheral levels (Pamenter & Hedge 1980, Rubello *et al.* 1992, Darras *et al.* 1999). In addition, in birds it has been established that glucocorticoids regulate thyroid function in an age-dependent manner, acting as inhibitors during post-hatch life and as stimulators during embryonic life (Darras *et al.* 1996). This occurs mainly by affecting the different peripheral deiodinases, which introduces another level of differential regulation, since these effects are not only age dependent, but also tissue dependent (Darras *et al.* 1996, Van der Geyten *et al.* 1999, 2001). In mammals, however, the situation is less clear. In adult humans, glucocorticoid administration results in a decrease in plasma T_3 levels together with an increase in rT_3 levels, whereas circulating T_4 generally remains unaffected, indicating that the overall effect is a shift from ORD to IRD (Duick *et al.* 1974, Chopra *et al.* 1975, Westgren *et al.* 1977, Lopresti *et al.* 1989), presumably through stimulation of D3. Although in adult rats plasma T_3 remains unaffected, T_3 metabolic clearance rate is reduced significantly in glucocorticoid- versus saline-treated animals

(Kaplan & Utiger 1978, Cavalieri *et al.* 1984, Jennings & Ferguson 1984). In contrast to humans, these changes are linked to a glucocorticoid-induced decrease in hepatic D1 activity (Balsam & Ingbar 1978). However, *in vitro*, a number of studies have demonstrated that glucocorticoids have the opposite effect and induce both D1 activity and D1 mRNA expression in rat hepatocytes (Menjo *et al.* 1993, Davies *et al.* 1996). So far it remains unclear as to what is the cause behind this discrepancy. Also in NRK-52E kidney cells, dexamethasone (DEX) increases D1 activity and mRNA expression (Davies *et al.* 1996), whereas in pituitary tumor cells DEX alone has no effect on D1 mRNA levels (Maia *et al.* 1995). Whether these observations, as in avian species, point to a tissue- or even an age-dependent effect of glucocorticoids on peripheral thyroid hormone metabolism remains to be investigated. For D2, such a differential regulation seems to exist. Recent *in vitro* data have demonstrated that both in rat and mouse pituitary cells D2 mRNA and activity are upregulated after glucocorticoid treatment (Kim *et al.* 1998, Araki *et al.* 2003), whereas in mouse HC11 mammary gland cells the opposite effects have been observed (Song & Oka 2003). Although glucocorticoids have been reported to downregulate D3 expression in rat brown fat vascular-stromal cells (Hernandez & St Germain 2002), to our knowledge these are the only data available concerning this topic in mammals.

The sometimes conflicting data between the different mammalian models used, including the discrepancies between the *in vivo* and *in vitro* systems, as well as the limited number of embryonic studies, make it hard to come to clear-cut conclusions concerning the role of glucocorticoids in the regulation of iodothyronine deiodination in mammals, especially during embryonic development. Due to the large variety in modes of administration and dosages used, comparison with data obtained in other well-studied species in this respect (e.g. the chicken) are also difficult and not always without bias. We therefore decided to investigate the acute effects of a single intravenous dose of DEX on circulating thyroid hormone levels and peripheral deiodinase activities in the developing rat. Although the rat is not always considered the best mammalian model in this respect (Darras *et al.* 1999, Wassen *et al.* 2004), it was our model of choice since most of the data concerning the regulation of iodothyronine deiodinases by glucocorticoids were obtained using the rat or rat-derived cell lines. We purposely limited the time-frame of our study to 2–4 h post-injection because we were primarily interested in the direct effects of glucocorticoids on thyroid hormone metabolism, and therefore wanted to avoid the possibility that changes in thyroid hormone levels would interfere with the glucocorticoid-induced changes in deiodinase activity. In addition, we decided to focus on day 20 of embryonic development (E20) and postnatal day 12 (P12), because from a developmental point of view these stages roughly delineate the time-frame during which develop-

ment (especially neural development) is extremely sensitive to thyroid hormones (Bernal 2002, Anderson *et al.* 2003). We also included postnatal day 5 (P5) because this stage corresponds to the human fetus or the chicken embryo just prior to birth or hatching respectively.

Materials and Methods

Animals

The Wistar (HsdCbp:WU) rats (Harlan, Horst, The Netherlands) used in these experiments were kept in a temperature (22 °C)- and light (14 h of light/day)-controlled room with free access to tap water and a standard diet. Fetal and neonatal rats were obtained by in-house breeding. For determination of the fetal age, the presence of a cervical plug in the morning was taken to be day 1 of pregnancy. Neonatal pups were kept with their mothers in separate cages, under identical conditions as before (see above). The University Ethical Committee for Animal Experiments approved all experimental animal manipulations.

Experimental design

For the experiments on E20 fetal rats, pregnant dams were weighed and anesthetized using 0.1 ml/100 g body weight Nembutal (sodium pentobarbital, 60 mg/ml). An incision was made in the abdominal cavity and part of the uterus, after which both the amniotic and chorionic membranes enveloping the fetuses were removed. DEX (Sigma-Aldrich, Bornem, Belgium) was dissolved in a small volume of ethanol (2% of final volume) and further diluted using 0.9% NaCl. Individual fetuses were injected via the vena umbilicalis with either 50 µl vehicle alone (control) or 5 µg DEX (± 1.5 µg/g body weight), a dose that corresponds to that used in previously reported rat (Cavalieri *et al.* 1984, Jennings & Ferguson 1984) and chicken studies (Darras *et al.* 1996, Van der Geyten *et al.* 1999, 2001). Two hours after this injection, blood was collected into heparinized tubes via the fetal vena subclavia and liver, brain and kidney were collected, immediately frozen in liquid nitrogen and stored at –80 °C. Plasma was stored at –20 °C. To avoid dehydration and hypothermia of the fetuses during the experiment, they were sprinkled at regular time-intervals with prewarmed 0.9% NaCl whereas body temperature of the pregnant dams and the fetuses was kept stable using an electrical heating pad. As an additional control (non-injected, NI), blood and tissue samples were also collected from E20 rat fetuses immediately following decapitation of the pregnant dams. Hence, these fetuses were not submitted to any experimental manipulation.

For the experiments using P5 and P12 rat pups, 50 µl vehicle (control) or 50 µg DEX (1.5–3 µg/g body weight

for P12 and P5 pups respectively) were injected intraperitoneally. To take into account the different mode of administration (intravenous versus intraperitoneal injection) between fetuses and pups, blood as well as tissues from P5 and P12 rat pups (killed by decapitation) were collected not at 2 h but at 4 h after the injection.

Due to the variable number of fetuses per mother, the number of samples analyzed per treatment group also varied. For E20 rats, this resulted in $n=16$ (two mothers) for the NI fetuses, $n=29$ (four mothers) for the saline-treated animals and $n=38$ (four mothers) for the DEX-treated rat fetuses. For the experiments with P5 pups (seven mothers), $n=34$ for both the saline- and DEX-treated groups. For the P12 experiment (six mothers), $n=28$ for the saline-treated pups and $n=29$ for the DEX-treated pups. In the latter experiments, half the pups from each mother were treated with DEX and the other half served as controls. In order to verify the data obtained, the entire study was repeated on an independent batch of animals, with $n=15$ for the NI fetuses, $n=20$ for the saline-treated animals and $n=20$ for the DEX-treated rat fetuses. For the experiments with P5 pups, $n=25$ for the saline-treated pups and $n=26$ for the DEX-treated pups. For the P12 experiment, $n=15$ for both the saline- and DEX-treated groups. This study confirmed the results from the first study, except for the D2 and D3 activities in P12 brain samples. Therefore, from the data obtained in the second study only the latter are discussed separately.

Thyroid hormone and deiodinase assays

T_4 and T_3 concentrations in plasma were determined by specific RIAs as defined previously (Van der Geyten *et al.* 2001).

Preparation of homogenates or microsomal fractions and measurement of D1, D2 and D3 activities were performed as described previously (Darras *et al.* 1992, 1996). In short, D1 activity was determined by measuring the radioiodide released from $[3',5'-^{125}\text{I}]\text{rT}_3$. Incubation mixtures contained 50 000 c.p.m. $[3',5'-^{125}\text{I}]\text{rT}_3$, 1 μM rT_3 , 100 μg protein/ml and 10 mM dithiothreitol (DTT) in sodium phosphate buffer (pH 7.2) and 2 mM EDTA. Reactions were stopped, and iodothyronines were precipitated by successive addition of 100 μl 5% bovine serum albumin and 500 μl 10% trichloroacetic acid at 0 °C. After centrifugation (3500 g, 10 min), 500 μl of the supernatant was counted in a γ -counter (Gammamaster, LKB Wallac, Erlangen, Germany). D2 activity was determined by measuring the radioiodide released from $[3',5'-^{125}\text{I}]\text{T}_4$. Incubation mixtures contained 50 000 c.p.m. $[3',5'-^{125}\text{I}]\text{T}_4$, 1 nM T_4 , 1 mg protein/ml, 100 nM T_3 , 0.1 mM PTU and 50 mM DTT in sodium phosphate buffer (pH 7.2) and 2 mM EDTA. The rest of the assay was identical to the D1 enzyme assay. To exclude possible interference from residual D1 activity, the ORD of T_4 was also measured in the presence of 100 nM T_4 . At this substrate concentration,

the D2 enzyme was saturated, so that all I^- measured in the assay was derived from the ORD of $[3',5'-^{125}\text{I}]\text{T}_4$ by D1. Only when I^- production was absent or minimal in the presence of 100 nM T_4 was the enzyme activity in the assay using 1 nM T_4 considered to be true D2 activity. For D3 activity, incubation mixtures contained 150 000 c.p.m. $[3'-^{125}\text{I}]\text{T}_3$, 10 nM T_3 (brain) or 1 nM T_3 (liver and kidney), 1 μM rT_3 , 125 μg protein/ml (liver), 250 μg protein/ml (brain) or 1 mg protein/ml (kidney), 0.1 mM PTU and 50 mM DTT in sodium phosphate buffer (pH 7.2) and 2 mM EDTA. Incubation was stopped by the addition of 300 μl ice-cold methanol. After centrifugation (3500 g, 10 min), 200 μl supernatant aliquots were transferred to vials containing 250 μl ammonium acetate buffer (0.02 M, pH 4.0). Labeled iodothyronines and I^- were separated by HPLC (Eelkman-Rooda *et al.* 1989). For this, 80 μl of the mixture was injected onto a C18 column (Nucleosil 100-5 C18, Filterservice, Eupen, Belgium) and eluted with a 28%/72% (v/v) mixture of acetonitrile and ammonium acetate buffer at a flow rate of 1 ml/min. Radioactivity was assessed with an on-line radioactivity monitor (LB 506 C-1; Perkin Elmer, Oosterhout, The Netherlands), and peaks were integrated using the RadioStar 3.0 software (Berthold Technologies, Bad Wildbad, Germany).

Plasma T_3 and T_4 levels are expressed as pmol/ml, whereas all activities were calculated as the amount of substrate deiodinated/mg protein per min.

Results

For the study on E20 rat fetuses, plasma T_3 (control: 0.748 ± 0.043 pmol/ml, DEX: 0.642 ± 0.041 pmol/ml, $P=0.121$) and T_4 (control: 39.87 ± 5.97 pmol/ml, DEX: 31.95 ± 5.16 pmol/ml, $P=0.219$) levels did not differ between the pregnant dams whose fetuses received saline or those whose fetuses were treated with DEX. In addition, the procedure (anesthetic+surgery) itself resulted in a decrease ($P<0.001$, ANOVA) in plasma T_4 levels (NI: 6.68 ± 0.44 pmol/ml, control: 3.61 ± 0.44 pmol/ml), kidney D1 activity (NI: 9.63 ± 0.26 pmol rT_3 /mg per min, control: 7.01 ± 0.52 pmol rT_3 /mg per min) and brain D2 activity (NI: 0.360 ± 0.015 fmol T_4 /mg per min, control: 0.228 ± 0.009 fmol T_4 /mg per min), whereas all other parameters measured remained unaffected.

In E20 rat fetuses, DEX administration had no effect on plasma T_3 and T_4 levels when compared with the vehicle (saline)-injected controls (Fig. 1A and B). In P5 rat pups, however, both T_3 and T_4 were increased 4 h after glucocorticoid treatment (Fig. 1C and D). This was again in contrast to P12 pups, in which plasma T_3 decreased significantly after DEX treatment, although circulating T_4 levels remained unaffected in comparison with the saline-treated controls (Fig. 1E and F).

Figure 1 Effects of intravenous administration of 5µg (E20, 2 h) or 50µg (P5 and P12, 4 h) DEX (Dex) on plasma (A, C and E) T₃ and (B, D and F) T₄ in (A and B) E20 rat fetuses and (C and D) P5 or (E and F) P12 rat pups. (Saline treated=Sal). Data are expressed as the means ± S.E.M. ($n_{E20,Sal}=29$, $n_{E20,Dex}=38$, $n_{P5,Sal}=34$, $n_{P5,Dex}=34$, $n_{P12,Sal}=28$, $n_{P12,Dex}=29$). ** $P<0.01$ and *** $P<0.001$. (unpaired two-tailed *t*-test).

In vitro hepatic D1 activity was decreased in DEX-treated rat fetuses (Fig. 2A), whereas postnatal glucocorticoid administration had no effect on hepatic D1 (Fig. 2C and E). Similarly to D1, *in vitro* D3 activity was decreased in the liver of DEX-treated rat fetuses (Fig. 2B), whereas

in both the P5 and P12 pups glucocorticoids increased hepatic D3 (Fig. 2D and F).

In accordance with the results in the liver, glucocorticoid administration resulted in a decreased D1 activity in the kidney of E20 fetuses (Fig. 3A), whereas in P5 and

Figure 2 Effects of intravenous administration of 5µg (E20, 2 h) or 50µg (P5 and P12, 4 h) DEX (Dex) on *in vitro* hepatic (A, C and E) D1 and (B, D and F) D3 activity, in (A and B) E20 rat fetuses and (C and D) P5 or (E and F) P12 rat pups. (Saline treated=Sal). Data are expressed as the means $\pm$ S.E.M. ($n_{E20,Sal}=29$, $n_{E20,Dex}=38$, $n_{P5,Sal}=34$, $n_{P5,Dex}=34$, $n_{P12,Sal}=28$, $n_{P12,Dex}=29$). * $P<0.05$ and *** $P<0.001$ (unpaired two-tailed *t*-test).

P12 kidneys DEX had no effect on *in vitro* D1 activity in comparison with control kidneys (Fig. 3C and E). Likewise, renal D3 was also increased in P5 and P12 treated rat pups (Fig. 3D and F), although in E20 fetuses

D3 activity remained unaffected when compared with the saline-injected controls (Fig. 3B).

In the brain, glucocorticoids induced an increase in *in vitro* D2 activity at both the E20 and P5 stages

Figure 3 Effects of intravenous administration of 5 μ g (E20, 2 h) or 50 μ g (P5 and P12, 4 h) DEX (Dex) on *in vitro* renal (A, C and E) D1 and (B, D and F) D3 activity, in (A and B) E20 rat fetuses and (C and D) P5 or (E and F) P12 rat pups. (Saline treated=Sal). Data are expressed as the means $\pm$ S.E.M. ($n_{E20,Sal}=29$, $n_{E20,Dex}=38$, $n_{P5,Sal}=34$, $n_{P5,Dex}=34$, $n_{P12,Sal}=28$, $n_{P12,Dex}=29$). * $P<0.05$ and *** $P<0.001$ (unpaired two-tailed *t*-test).

(Fig. 4A and C). At P12, D2 decreased following DEX administration (Fig. 4E), although this could not be confirmed in the duplicate study where D2 remained unaltered by glucocorticoids at this developmental

stage (control: 0.532 ± 0.045 fmol T_4 /mg per min, DEX: 0.514 ± 0.027 fmol T_4 /mg per min). For D3, the situation was somewhat more complex. In E20 fetuses, DEX increased *in vitro* D3 activity (Fig. 4B), whereas

Figure 4 Effects of intravenous administration of 5µg (E20, 2 h) or 50µg (P5 and P12, 4 h) DEX (Dex) on *in vitro* brain (A, C and E) D2 and (B, D and F) D3 activity, in (A and B) E20 rat fetuses and (C and D) P5 or (E and F) P12 rat pups. (Saline treated=Sal). Data are expressed as the means ± s.e.m. ($n_{E20,Sal}=29$, $n_{E20,Dex}=38$, $n_{P5,Sal}=34$, $n_{P5,Dex}=34$, $n_{P12,Sal}=28$, $n_{P12,Dex}=29$). * $P<0.05$ and *** $P<0.001$ (unpaired two-tailed *t*-test).

it decreased D3 in P5 pups (Fig. 4D). Finally, in P12 pups DEX had no effect on brain D3 (Fig. 4F). The latter result, however, was again different in the duplicate study, where D3 activity showed a moderate,

albeit significant increase, after DEX administration to P12 rat pups (control: 10.39 ± 0.36 fmol T₃/mg per min, DEX: 11.85 ± 0.46 fmol T₃/mg per min, $P<0.05$).

Discussion

The most striking result of the present study was that glucocorticoids affected circulating thyroid hormone levels as well as peripheral deiodination differently at all developmental stages studied. That in E20 rat pups circulating T_3 and T_4 levels were not affected by DEX despite the significant changes in hepatic and renal deiodination, could be the result of maternal hormone levels masking potential changes in fetal plasma thyroid hormone concentrations. However, at E20 the rat fetal thyroid is already fully active and maternal T_4 only accounts for 17.5% of the fetal extrathyroidal T_4 pool (Morreale de Escobar *et al.* 1990, 2004), thereby indicating that only minor changes in fetal plasma T_3 and T_4 would be masked by the unchanged maternal levels. Taken together, these data therefore suggested that in the days just prior to parturition the control of circulating thyroid hormone levels seems more dependent on fetal thyroidal secretion than on peripheral deiodination, as is generally assumed to be the case. In contrast to the fetal situation, DEX injection increased both plasma T_3 and T_4 in P5 rat pups and this without affecting hepatic and renal D1. Although peripheral T_3 production did not seem to be affected by glucocorticoids at this stage, the increase in thyroidal T_4 secretion alone together with an unaltered D1 activity was probably sufficient to account for the increase in circulating T_3 levels. In P12 pups, however, DEX no longer affected circulating T_4 levels, whereas it significantly decreased plasma T_3 concentrations. Although previous experiments, using both adult and P7 rats, indicated that glucocorticoids reduced hepatic T_3 production (Jennings & Ferguson 1984, McCann *et al.* 1984), our results suggested that in P12 rat pups the DEX-induced decrease in plasma T_3 levels is more likely due to the increased hepatic and possibly also renal T_3 degradation (via D3) without changes in peripheral T_3 production. A similar mechanism has been proposed to explain the glucocorticoid-induced decrease in plasma T_3 levels in man, although so far no direct experimental proof has been provided for this hypothesis (Chopra *et al.* 1975, Westgren *et al.* 1977, Lopresti *et al.* 1989).

Also in the brain, thyroid hormone metabolism was differentially affected by glucocorticoids depending on the developmental stage at which exposure occurred. At E20, the rise in brain D2 suggested a possible glucocorticoid-induced increase in intracellular T_3 availability in this tissue. That brain D3 activity also increased simultaneously might indicate that for the brain this increase comes at the wrong time in development and therefore the brain, through induction of the IRD pathway, takes protective measures to counteract this premature increase in local T_3 levels. This clearly differs from that which occurs in the brain of P5 rat pups, where the increased plasma T_3 and T_4 levels, in combination with the opposing effects of DEX on brain D2 and D3, indicated that in this tissue and at this

developmental stage glucocorticoids favor thyroid hormone activation by stimulating the ORD of T_4 and inhibiting the IRD of T_3 . Similar results have been found in embryonic birds (Darras *et al.* 1996, Van der Geyten *et al.* 2001) and larval amphibians (Darras *et al.* 2002). Since it has been well established that the brain of early neonatal rats is developmentally equivalent to the 2nd trimester human fetal brain and to the brain of late embryonic chickens (Sedláček 1972, Markus *et al.* 1987, Rostas *et al.* 1991, Bernal 2002), these results suggested that this glucocorticoid-induced thyroid hormone activation in the brain might represent a development-related and important maturing mechanism that is present across vertebrates. However, at P12, glucocorticoids no longer stimulated brain D2 activity, thereby indicating that glucocorticoids no longer push brain thyroid hormone metabolism towards increasing local T_3 levels. This situation probably also persists further in life, since also in P21 pups (McCann *et al.* 1984) as well as in adult rats (Anguiano *et al.* 1995) glucocorticoids no longer increase brain D2 activity.

This transient window in time (between E20 and P12) during which rat brain thyroid hormone activity appears to be stimulated by glucocorticoids coincides with some important neurodevelopmental processes, such as neuronal migration, oligodendrocyte development and cerebellar maturation. For example, dendritic arborization of cerebellar Purkinje cells is only sensitive to thyroid hormones during the first 2 weeks postnatal (Anderson *et al.* 2003), which corresponds to the time-frame during which glucocorticoids stimulate brain thyroid hormone activity. In addition, in the visual cortex, the hypothyroid-induced changes in distribution and number of dendritic spines in pyramidal cells can only be reversed when thyroid hormone replacement is initiated before day 12, whereas a delay beyond postnatal day 12 results in abnormal adult pyramidal cells (Anderson *et al.* 2003). These observations indicated that not only thus does there exist a narrow window in time during which brain development is thyroid hormone sensitive (Bernal 2002, Anderson *et al.* 2003), but this window in time largely overlaps with the period during which glucocorticoids stimulate brain T_3 availability through opposing effects on brain D2 and D3 activity.

In addition, the current results also demonstrated that D3 is not only regulated in an age-dependent manner by glucocorticoids, but also in a tissue-specific manner. This, however, is less clear for D1 and D2. We did not find a difference between the effects of DEX on hepatic and renal D1 activity at any of the developmental stages studied. However, Maia *et al.* (1995) demonstrated, in pituitary tumor cells, that DEX alone had no effect on D1 mRNA expression, whereas it enhanced T_3 -stimulated D1 mRNA levels. Although this may point to some degree of tissue specificity, it might also be an observation that is only relevant for *in vitro* systems. Indeed, in cultured

liver and kidney cells also, glucocorticoids alone or in combination with T_3 increase D1 mRNA and/or activity (Davies *et al.* 1996, Menjo *et al.* 1993, Yamaguchi *et al.* 1999). So far, the mechanisms underlying the discrepancies between the *in vivo* and *in vitro* effects of glucocorticoids have not been elucidated, so at the moment the question concerning the tissue-specific effects of DEX on D1 remains unanswered. Similarly, the question as to whether glucocorticoids have tissue-specific effects on D2 or not cannot be answered based on the presented data alone. Recent reports have indicated that in rat (GH3 and GC cells) and mice (AtT-20 cells) pituitary cells D2 mRNA levels are also increased after glucocorticoid treatment (Kim *et al.* 1998, Araki *et al.* 2003), whereas in mouse HC11 (mammary gland) cells DEX decreases D2 activity, D2 mRNA levels and D2 promoter activity (Song & Oka 2003). Although these data could be indicative for tissue-specific regulation, additional *in vivo* experiments will be required if conclusive evidence is to be obtained.

In conclusion, the presented data have demonstrated that, in the developing rat, glucocorticoids regulate peripheral thyroid hormone metabolism in both a tissue- and age-dependent manner. In E20 rat fetuses, glucocorticoids had no effects on circulating thyroid hormone levels despite their clear effects on hepatic and renal deiodinases, thereby indicating that under these conditions circulating thyroid hormone levels are more dependent on thyroidal secretion than on peripheral deiodination. In contrast, in P5 rat pups, DEX did not seem to have any effects on hepatic and renal T_3 production (via D1), whereas D3 activity in both these tissues increased significantly. These observations therefore suggested that the DEX-induced increase in circulating T_3 levels is a direct consequence of the increase in plasma T_4 levels. In P12 pups, however, the main effect of glucocorticoids on circulating T_3 levels is by increasing the IRD pathway through induction of D3 in both liver and kidney. Finally, in the brain, glucocorticoids stimulate thyroid hormone activity only during a short period of time (between E20 and P12) that largely overlaps with the transient window in time during which brain development is thyroid hormone sensitive.

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