Influence of maternal hyperthyroidism in the rat on the expression of neuronal and astrocytic cytoskeletal proteins in fetal brain

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Abstract

Maternal hypothyroidism during pregnancy impairs brain function in human and rat offspring, but little is known regarding the influence of maternal hyperthyroidism on neurodevelopment. We have previously shown that the expression of neuronal and glial differentiation markers in fetal brain is compromised in hypothyroid rat dam pregnancies and have now therefore extended this investigation to hyperthyroid rat dams. Study groups comprised partially thyroidectomised dams, implanted with osmotic pumps infusing either vehicle (TX dams) or a supraphysiological dose of thyroxine (T4) (HYPER dams), and euthyroid dams infused with vehicle (N dams). Cytoskeletal protein abundance was determined in fetal brain at 21 days of gestation by immunoblot analysis. Relative to N dams, circulating total T4 levels were reduced to around one-third in TX dams but were doubled in HYPER

dams. Fetal brain weight was increased in HYPER dams, whereas litter size and fetal body weight were reduced in TX dams. Glial fibrillary acidic protein expression was similar in HYPER and TX dams, being reduced in both cases relative to N dams. α -Internexin (INX) abundance was reduced in HYPER dams and increased in TX dams, whereas neurofilament 68 (NF68) exhibited increased abundance in HYPER dams. Furthermore, INX was inversely related to – and NF68 directly related to – maternal serum total T4 levels, independently of fetal brain weight. In conclusion, maternal hyperthyroidism compromises the expression of neuronal cytoskeletal proteins in late fetal brain, suggestive of a pattern of accelerated neuronal differentiation.

Journal of Endocrinology (2002) **175**, 597–604

Introduction

In humans and rats, maternal thyroid hormone (TH), predominantly thyroxine (T4), is transferred to the fetus from before the onset of fetal thyroid function. Transferred T4 accumulates in the fetal brain, and is deiodinated to bioactive 3,5,3'-tri-iodothyronine (T3) (Morreale de Escobar *et al.* 1997, Pickard *et al.* 1997). T3 nuclear receptors (TR) are expressed in early fetal brain, therefore maternal TH may regulate fetal brain development (Morreale de Escobar *et al.* 1997, Pickard *et al.* 1997). Indeed, children born to hypothyroxinaemic mothers in iodine-deficiency endemias are at increased risk of a spectrum of neurological disorders. Such damage appears to be sustained in the first trimester of pregnancy, before the onset of fetal TH secretion (Connolly & Pharoah 1989, Morreale de Escobar *et al.* 1997), and measures of cognitive and motor function in offspring correlate with maternal serum total and free T4 levels. In iodine-replete environments, cognitive and motor development is also impaired in offspring of women with moderate hypothyroidism (Man *et al.* 1991, Haddow *et al.* 1999) or even

low-normal serum free T4 levels (Pop *et al.* 1999). Furthermore, psychomotor test scores of infants are directly related to first trimester maternal serum free T4 levels (Pop *et al.* 1999).

In the rat, moderate maternal hypothyroidism also results in deficient locomotor and learning behaviour in the progeny (Attree *et al.* 1992, Porterfield & Hendrich 1993), and brain development is disrupted from before the onset of fetal thyroid function (Pickard *et al.* 1993, 1999). Early neuronal maturation, in particular, appears compromised (Evans *et al.* 1999, Sampson *et al.* 2000), with some deficits persisting in postnatal life (Porterfield & Hendrich 1991, Pickard *et al.* 1997, Evans *et al.* 1999).

Most studies on the influence of the maternal thyroid on fetal development have centered on hypofunction, mainly because this condition is more commonly encountered during pregnancy than hyperfunction. Nevertheless, maternal hyperthyroidism is a significant endocrinological disorder in pregnancy; the prevalence of thyrotoxicosis (predominantly Graves' disease) being 0.05–0.2, with a further 3% of mothers exhibiting gestational transient thyrotoxicosis (Burrow 1993, Glinoe 1997). Maternal TH

excess during pregnancy results in an increased incidence of pre-eclampsia and low birth weight, the latter being due to premature delivery (Millar *et al.* 1994), but little is known regarding its effects on neurodevelopment. Two early studies independently reported a high incidence of hyperthyroidism in mothers of schizophrenics (Pollin *et al.* 1966, MacSweeney *et al.* 1978); however, these findings remain to be confirmed.

In rats, chronic maternal hyperthyroidism stimulates fetoplacental and neonatal growth (Spencer & Robinson 1993), but its effects on fetal brain have not been studied. Induction of maternal hyperthyroidism during late pregnancy also increases fetal body weight, but fetal brain growth and cerebellar development appear normal (Schwartz *et al.* 1997). Nevertheless, the expression of several neuronal genes (neuroendocrine-specific protein and Oct1) in fetal cerebral cortex are sensitive to acute maternal T4 administration during late gestation (Dowling *et al.* 2000).

There is evidence to suggest that TH regulates specific markers of neuronal and glial differentiation, such as neurofilament-68 (NF68) and glial fibrillary acidic protein (GFAP), in both *in vivo* and *in vitro* models of congenital hypothyroidism (Gould *et al.* 1990, Lima *et al.* 1997, Ghosh *et al.* 1999, Rahamam *et al.* 2000). Recently, we have shown that maternal hypothyroidism can also disturb the ontogeny of these proteins in fetal brain, as well as that of α -internexin (INX) – the earliest appearing neuronal specific intermediate filament (Sampson *et al.* 2000). In the present study, we have investigated the hypothesis that chronic moderate maternal hyperthyroidism also impairs neuronal and astrocytic differentiation.

Materials and Methods

Materials

Alzet osmotic pumps (Model 2004, pumping rate 0.25 μ l/h for 28 days) were purchased from Charles River UK (Margate, Kent, UK). Bovine serum albumin (BSA), T4 and monoclonal antibodies to GFAP (clone GA-5), vimentin (clone V9) and NF68 (clone NR-4) were purchased from Sigma-Aldrich Chemical Company (Gillingham, Dorset, UK). Monoclonal antibody to INX (clone 3 G8) was purchased from Chemicon International (Harrow, UK). Western blotting reagents were purchased from Amersham Bioscience (Little Chalfont, Bucks, UK). The total T4 radioimmunoassay (RIA) kit was obtained from Skybio (Wyboston, Beds, UK), the total T3 RIA kit from B M Browne Ltd (Reading, Berks, UK) and the rat thyroid-stimulating hormone (TSH) RIA kit from Biocode Biotechnology (Liege, Belgium).

Animal model

All animal procedures were performed in accordance with the Animals (Scientific Procedures) Act 1986. Sprague–

Dawley rat dams were partially thyroidectomised by surgery (Evans *et al.* 1999), allowed to recover for 2 weeks, then subcutaneously implanted posterior to the scapulae with an osmotic minipump, infusing either 1.5 μ g T4/100 g pre-mating body weight/day (HYPER dams) or vehicle (TX dams). Age-matched normal dams, implanted with pumps infusing vehicle (N dams), were also studied. T4 was dissolved in 30 mM NaOH and, after spectrophotometric verification of the concentration (absorbance at 325 nm), diluted in phosphate-buffered saline (PBS; pH 7.4) containing 5% (w/v) BSA; vehicle therefore consisted of an equivalent amount of 30 mM NaOH in PBS/BSA. After a 2-day recovery period, dams were time-mated with normal males. Dams were tail bled for TH determination before osmotic pump implantation and within 3 days after mating. All animals were maintained at 22 °C on a cycle of 14 h light:10 h darkness, with free access to a standard small laboratory animal diet; the drinking water of TX and HYPER animals was supplemented with 0.1% (w/v) calcium lactate. At 21 days of gestation (dg), pregnant dams were stunned and killed by cervical dislocation, and a cardiac blood sample was taken for serum TH assay. Uterine horns containing fetoplacental units were removed to ice and maternal carcass weight was determined. Feto-placental tissues were dissected on ice, weighed, snap-frozen on dry ice then stored at –20 °C.

Sample preparation and Western blotting

Whole fetal brains were pooled from each pregnancy ($n \geq$ three brains/pool), homogenised in 9 vol ice-cold TE buffer (2 mM EDTA, 0.5% (v/v) Triton X-100 and 10 mM phenylmethylsulphonylfluoride in 50 mM Tris-HCl; pH 6.8) and subjected to Western blot analysis (Sampson *et al.* 2000). Briefly, fetal brain homogenates from N, HYPER and TX dams were electrophoresed side-by-side on SDS-PAGE gels along with the appropriate standard (2.5 μ g protein from normal 16 dg fetal brain for vimentin; 0.25, 0.5 and 2.5 μ g protein from normal adult brain for GFAP, NF68 and INX respectively) and molecular weight markers, blotted onto nitrocellulose membranes and probed with specific monoclonal antibodies. Detection was via a horseradish peroxidase-linked secondary antibody and ECL reagents (Amersham, following the manufacturer's protocol). Autoradiographic images were digitised and signals analysed using NIH Image 6.1 (US National Institutes of Health; <http://rsb.info.nih.gov/ni-image/>). Sample signals were expressed relative to the appropriate filter standard to correct for filter-to-filter variation.

Determination of maternal thyroid status

TSH, total T3 and total T4 levels were determined in maternal serum by RIA, according to the manufacturers' protocols. The T3 antibody does not cross react with any

major analogues of T3. The T4 antibody exhibits cross reactivity of less than 2.5% with T3 and 30% with reverse T3 in molar % terms.

Determination of DNA and protein

DNA was determined by a fluorimetric method (Labarca & Paigen 1980), and protein by Coomassie dye-binding (Bradford 1976).

Statistical analysis

All values are means $\pm$ S.E.M. Statistical significance ($P < 0.05$) was determined by analysis of variance (ANOVA) with *post-hoc* analysis by Fisher's PLSD test. Where necessary, data were transformed prior to analysis to satisfy the criteria for ANOVA. Linear and non-linear regression analyses were performed as appropriate, with a runs test to determine data linearity. Statistical analyses were performed using Statview 1.03 (Abacus Concepts, Inc., Berkeley, CA, USA) and GraphPad Prism 2.0c (GraphPad Software, Inc., San Diego, CA, USA).

Results

Maternal thyroid status

Serum total T4 levels in HYPER dams during early gestation (0–3 dg) were more than double those in N dams, whereas they were reduced in TX dams to approximately one-third of those in N dams (Table 1). These relationships were maintained at 21 dg. Serum total T3 levels were also increased in HYPER dams but only during early gestation, whereas they were normal in TX dams at all stages of pregnancy (Table 1). In early pregnancy, serum TSH was undetectable in HYPER dams and was markedly increased in TX dams relative to N dams (Table 1). Near term, however, serum TSH levels were normal in HYPER dams but remained elevated in TX dams.

Reproductive performance and feto-placental growth

At 21 dg, maternal carcass weight was equivalent in HYPER dams (354.8 ± 11.3 g; $n=6$), TX dams (340.5 ± 10.4 g; $n=4$) and N dams (357.9 ± 20.4 g; $n=6$). Litter size and fetal body weight were also normal in HYPER dams, whereas these parameters were reduced in TX dams (Table 1), as expected (Sampson *et al.* 2000). In contrast, fetal brain weight was slightly elevated (by 9%) in HYPER dams relative to N dams, but normal in TX dams (Table 1). Fetal brain weight exhibited a linear relationship with the maternal serum T4 level at 21 dg ($r=0.65$, $P < 0.01$) or during early gestation ($r=0.71$, $P < 0.005$). Weights of the placenta (whole, and after

Table 1 Maternal serum thyroid hormone levels, litter size and fetal body and brain weight in normal (N), partially thyroidectomised (TX) and hyperthyroid (HYPER) rat dam pregnancies. Values are means $\pm$ S.E.M. ($n \geq 4$; $*n=3$)

	Early gestation			Post mortem (21 days of gestation)			Litter size	Body weight (g)	Brain weight (mg)
	T4 (nM)	T3 (nM)	TSH (ng/ml)	T4 (nM)	T3 (nM)	TSH (ng/ml)			
Dam									
N	53.2 ± 4.7	0.75 ± 0.10	5.8 ± 1.8	25.7 ± 3.4	0.82 ± 0.28	$2.2 \pm 0.3^*$	13.5 ± 1.1	5.29 ± 0.14	195 ± 5
TX	18.7 ± 2.4^b	0.68 ± 0.21	37.6 ± 10.1^b	4.6 ± 2.2^b	0.47 ± 0.08	31.7 ± 10.3^b	7.8 ± 1.1^b	4.33 ± 0.32^b	182 ± 7
HYPER	$122.1 \pm 17.5^{a,c}$	$1.63 \pm 0.27^{a,c}$	ND	$77.5 \pm 8.0^{a,c}$	0.82 ± 0.19	1.8 ± 0.3^c	10.5 ± 1.2	5.50 ± 0.07	$212 \pm 4^{a,c}$

ND, below the TSH assay detection limit (0.1 ng/ml).

^a $P < 0.05$ HYPER vs N; ^b $P < 0.05$ TX vs N; ^c $P < 0.05$ HYPER vs TX.

Figure 1 Maternal thyroid status and vimentin and GFAP expression in fetal brain. Protein abundance of (A) vimentin and (B) GFAP in 21 dg fetal brain from N (shaded bars), TX (open bars) and HYPER (solid bars) dam pregnancies. Values are means $\pm$ S.E.M. ($n \geq 4$). ^a $P < 0.05$ HYPER vs N; ^b $P < 0.05$ TX vs N.

dissection into maternal and fetal sides) and fetal liver, lung and heart were normal in HYPER and TX dams (data not shown). Fetal brain protein and DNA concentrations and protein:DNA ratios were also normal in HYPER and TX dams (data not shown).

Cytoskeletal protein abundance in fetal brain

All antibodies specifically recognised protein species of the expected size, as previously reported (Sampson *et al.* 2000). The abundance of vimentin, an intermediate filament protein which is expressed mainly in neural precursor cells in fetal brain, was similar in HYPER, TX and N dams (Fig. 1A). In contrast, GFAP, which is the predominant intermediate filament protein of mature astrocytes, exhibited decreased abundance in HYPER dams (by 49%) and in TX dams (by 62%) relative to N dams (Fig. 1B).

Figure 2 Maternal thyroid status and INX expression in fetal brain. (A) Protein abundance of INX in 21 dg fetal brain from N (shaded bars), TX (open bars) and HYPER (solid bars) dam pregnancies. Values are means $\pm$ S.E.M. ($n \geq 4$). ^a $P < 0.05$ HYPER vs N; ^b $P < 0.05$ TX vs N; ^c $P < 0.05$ HYPER vs TX. (B) Regression of INX protein abundance in fetal brain from N ($\square$), TX (Δ) and HYPER ($\circ$) dams, against maternal serum total T4 at 21 dg ($r = -0.78$, $P < 0.001$).

The expression of two markers of early neuronal differentiation was also studied: INX, an axonal type IV intermediate filament protein, which is expressed in newly differentiating neurons, and NF68, the low molecular weight neurofilament triplet protein, which is expressed subsequent to INX. The abundance of INX was reduced (by 40%) in HYPER dams, but increased (by 69%) in TX dams, when compared with N dams (Fig. 2A). Upon combining data from all three animal groups, INX protein abundance was found to be inversely related to maternal serum T4 at 21 dg ($r = -0.78$, $P < 0.001$; Fig. 2B) or during early gestation ($r = -0.79$, $P < 0.001$). In contrast,

Figure 3 Maternal thyroid status and NF68 expression in fetal brain. (A) Protein abundance of NF68 in 21 dg fetal brain from N (shaded bars), TX (open bars) and HYPER (solid bars) dam pregnancies. Values are means $\pm$ S.E.M. ($n \geq 4$). ^a $P < 0.05$ HYPER vs N; ^b $P < 0.05$ HYPER vs TX. (B) Regression of NF68 protein abundance in fetal brain from N ($\square$), TX ($\triangle$) and HYPER ($\circ$) dams, against maternal serum total T4 at 21 dg ($r = 0.61$, $P < 0.05$).

the abundance of NF68 was elevated (by 44%) in HYPER dams but unchanged in TX dams relative to N dams (Fig. 3A). Furthermore, NF68 abundance in the combined data set was directly related to maternal serum T4 at 21 dg ($r = 0.61$, $P < 0.05$; Fig. 3B) or during early gestation ($r = 0.78$, $P < 0.001$).

In fetal brain from N dams, protein abundance of GFAP and NF68 increases during late gestation whereas that of INX declines (Sampson *et al.* 2000). Near term fetal brain weight is increased in HYPER dams, so that the changes in INX and NF68 abundance may occur secondary to enhanced fetal brain growth. Indeed in the combined data, INX abundance was inversely related to fetal brain weight ($r = -0.65$, $P = 0.007$), and NF68 abundance tended

towards a direct relationship ($r = 0.44$, $P = 0.091$). This potentially confounding effect of fetal brain weight was further examined by multiple regression analysis. Regression of INX abundance on fetal brain weight and maternal serum total T4, either at 21 dg or during early gestation, yielded similar significant relationships (multiple regression coefficient $R = 0.801$ and 0.795 respectively, $P < 0.002$; $n = 16$). In both models, however, INX was related only to maternal serum T4 ($P < 0.020$) and not to fetal brain weight. Regression of NF68 on fetal brain weight and maternal serum T4 also gave significant relationships, albeit this relationship was stronger in the model with early gestation T4 levels ($R = 0.794$, $P = 0.002$; $n = 16$) than in the model with T4 levels at 21 dg ($R = 0.616$, $P = 0.045$; $n = 16$). Indeed, whereas the NF68 was related to early gestation maternal serum T4 ($P = 0.014$), it only tended towards a significant relationship with maternal serum T4 at 21 dg ($P = 0.068$). Nevertheless, NF68 abundance was unrelated to brain weight in both models.

Discussion

We postulated that chronic maternal hyperthyroidism disrupts fetal brain development and developed a rat dam model to test this hypothesis. The rat placenta is only a limited barrier to the transfer of maternal T4 and T3 (Calvo *et al.* 1992), but the fetal brain is dependent upon local deiodination of T4 for its T3 supply (Ruiz de Ona *et al.* 1988). Consequently T4 was used to induce maternal hyperthyroidism in the model. This was administered by continuous infusion to avoid wide fluctuations in circulating maternal T4 levels resulting from daily injection regimes (Spencer & Robinson 1993). Infusion of T4 into TX dams increased circulating maternal T4 levels to more than double those of N dams throughout pregnancy; maternal serum T3 levels were also increased, but only during early gestation (0–3 dg). Furthermore, maternal serum TSH levels in HYPER dams were undetectable in early gestation, and depressed relative to TX dams alone at 21 dg. Thus HYPER dams were moderately hyperthyroid during early gestation but hyperthyroxinaemic during late gestation. The latter findings agree with previous studies of hypothyroid rat dams infused with T4 during late gestation (Calvo *et al.* 1990, 1992). However, a similar T4 dose to the one used in the present study was only sufficient to normalize circulating total T4 levels in the previous studies. A possible explanation for this discrepancy may be that the dams receiving T4 in the previous studies were made hypothyroid by methimazole (MMI) administration, whereas those in the current study were surgically thyroidectomised. MMI increases thyroidal type I deiodinase activity (Erickson *et al.* 1982) and may therefore facilitate clearance of circulating T4. In addition, removal of the majority of the thyroid gland is likely to disturb TH homeostatic mechanisms. Indeed, in preliminary

experiments, an equivalent dose of T4 infused into N dams failed to consistently increase circulating total T4 levels to the same degree.

Infusion of T4 prevented the reductions in litter size and fetal body weight seen in TX dams near term, these parameters being unchanged in HYPER dams relative to N dams. More severe, chronic maternal hyperthyroidism is also without effect on litter size, but increases fetoplacental weight near term (Spencer & Robinson 1993). Similarly, T4 given during late gestation stimulates fetal growth in severe (Schwartz *et al.* 1997), but not moderate, hyperthyroidism (Calvo *et al.* 1992, Morreale de Escobar *et al.* 1993). Thus it appears that only severe maternal hyperthyroidism influences general fetoplacental growth. In the current study, fetal brain weight was marginally higher in HYPER dams relative to N and TX dams, but the weights of other fetal tissues examined were normal, suggesting that maternal hyperthyroidism may have selective effects on the central nervous system. In contrast, severe maternal hyperthyroidism induced from 15 dg has no effect on fetal brain weight, even though it increases fetal body weight (Schwartz *et al.* 1997). A critical period may therefore exist during early gestation when brain growth is specifically sensitive to maternal hyperthyroidism.

The main finding was that moderate maternal hyperthyroidism resulted in abnormal expression of neuronal cytoskeletal proteins in fetal brain near term. Thus, the abundance of INX was decreased, whereas that of NF68 was increased in HYPER dams relative to N and TX dams. In TX dams, INX abundance was increased relative to N dams, as expected (Sampson *et al.* 2000). However, NF68 abundance in TX dams was unchanged in the present study, whereas it was reduced in the earlier study (Sampson *et al.* 2000). This discrepancy may be due to the more severe degree of maternal hypothyroidism in the earlier study (pre-mating maternal serum total T4 levels in TX dams were around half those in the present study), which was sufficient to retard fetal brain growth near term (Sampson *et al.* 2000). In this regard, regression analysis of the combined N, TX and HYPER dam data revealed that NF68 protein abundance was directly related to – and INX protein abundance was inversely related to – maternal serum T4 levels, with INX exhibiting a greater dependency than NF68 on maternal serum T4. Importantly, these relationships were independent of the more general effects of maternal thyroid status on fetal brain growth. Since the abundance of INX declined and that of NF68 increased during late fetal brain development, these findings indicated that maternal hypothyroidism delays, whereas maternal hyperthyroidism accelerates, neuronal maturation.

TH action in the developing brain is thought to be mediated via TR-dependent regulation of target gene transcription (Koibuchi & Chin 2000). INX and NF68 are unlikely to be primary targets for maternal TH action

however, since severe maternal hypothyroidism has no effect on their transcript levels in fetal brain, even prior to the onset of fetal thyroid function (Sampson *et al.* 2001). Nevertheless, T3 acutely stimulates INX protein expression in primary cultures of early differentiating rat neurons (Sampson *et al.* 2002), and maternal hypothyroidism reduces INX protein abundance in fetal brain prior to the onset of fetal thyroid function (Sampson *et al.* 2000), supporting a regulatory role for maternal TH in early neuronal development. From around 17 dg, however, the fetal brain can maintain its intracellular T3 level despite wide fluctuations in maternal T4 levels (Morreale de Escobar *et al.* 1992). Thus, this direct action on INX abundance by T3 is not likely to be the cause of the changes reported here. Rather, they may reflect chronic alterations in the timing of neuronal differentiation sustained during early gestation. This is supported by the divergent effects of severe maternal hypothyroidism on INX and NF68 abundance before and after the onset of fetal TH synthesis (Sampson *et al.* 2000). Moreover, this postulate may explain why NF68 and INX abundance in late fetal brain exhibit linear relationships with maternal serum T4, despite the operation of brain T3 homeostatic mechanisms.

Earlier work has shown that maternal hypothyroidism reduces GFAP protein in fetal brain in late gestation (Sampson *et al.* 2000). Present results agree with this finding; however, GFAP abundance in HYPER and TX dams was reduced by a similar extent when compared with N dams. This is in contrast to *in vivo* and *in vitro* models of congenital hypothyroidism in which T3 induces brain GFAP expression in an age- and region-specific manner (Gould *et al.* 1990, Lima *et al.* 1997). Another study, however, found that T3 treatment of astroglial primary culture from mouse neocortex and cerebellum decreased GFAP expression (Andres-Barquin *et al.* 1994). Thus, in our studies of whole brain, it is possible that regions with increased GFAP expression have been masked by others with quantitatively larger decreases. In addition, the previous studies were reporting a direct effect of T3 on GFAP expression. For reasons discussed earlier, the changes in GFAP expression in the current study are likely to represent an indirect effect arising from an earlier insult – and this may also account for the discrepancies. Another alternative is that the T4 infusion is unable to prevent GFAP compromise in TX dams. Further work is therefore required to determine whether normalisation of circulatory T4 levels in TX dams can prevent the disturbance of fetal brain GFAP protein expression.

Thus, while maternal hypothyroidism tends to delay, in general, neural cell differentiation, the effects of maternal hyperthyroidism are more complex – neuronal and glial differentiation are accelerated and delayed respectively. Given the importance of neuronal–glial interactions for the establishment of vital neural connections, this discordant response to maternal hyperthyroidism is potentially more

damaging to brain development than a general delay or acceleration. Studies using postnatal progeny of hyperthyroid dams are needed, however, to assess the long-term consequences of this disruption. As well as being markers of neuronal differentiation, both INX and NF68 are thought to be involved in the maintenance and stability of neuronal axons (Kirkpatrick & Brady 1999). The role of GFAP is less clear, although it has been implicated in neuronal–glial interactions (McCall *et al.* 1996). Thus alterations in the levels of these proteins *per se* may result in disturbed neuron–neuron and neuron–glia connectivity; whether the changes reported in the current study are sufficient to cause these effects is, however, unknown and requires investigation by immunohistological studies.

Several important differences exist between rats and humans which must be taken into account when considering the relevance of these findings for humans: for example, materno–fetal TH economy and the maturity of the brain at birth (Fukuda *et al.* 1980, Porterfield & Hendrich 1993, Glinoe 1997). Nevertheless, in both species, materno–fetal T4 transfer occurs throughout pregnancy, and brain development is comparable prior to the onset of fetal thyroid activity, as are many elements of brain TH metabolism and receptor expression (Porterfield & Hendrich 1993, Morreale de Escobar *et al.* 1997). Interestingly, parameters of cognitive and motor function in human offspring exhibit linear relationships with circulating maternal total and free T4 levels during early gestation (Connolly & Pharoah 1989, Pop *et al.* 1999). Given the results of the present study, it is possible that maternal TH regulation of neuronal differentiation underlies such relationships. Furthermore, they indicate that even moderate maternal hyperthyroidism is detrimental to brain development. However, these points require detailed clinical investigation.

In conclusion, maternal hyperthyroidism disrupts the expression of neuronal cytoskeletal proteins in late fetal brain. Furthermore, the changes tend to be the converse of those seen in maternal hypothyroidism and are indicative of accelerated neuronal differentiation. Work is in progress to determine the regional localisation of such effects and to further delineate the critical period of dependency of neuronal and astrocytic differentiation on maternal thyroid status.

Acknowledgement

This work was funded by a grant from Action Research.

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Received in final form 28 August 2002

Accepted 5 September 2002