Effect of growth hormone on growth and myelination in the neonatal hypothyroid rat

R. A. King, R. M. Smith, D. J. Meller, G. W. Dahlenburg* and J. D. Lineham

CSIRO (Australia), Division of Human Nutrition, Kintore Avenue, Adelaide, South Australia 5000, Australia
*Department of Pediatrics, University of Adelaide, Queen Victoria Hospital, Rose Park, South Australia 5067, Australia

(J. D. Lineham is now at Baker Medical Research Institute, Commercial Road, Prahran, Victoria 3181, Australia)

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ABSTRACT

The possible involvement of a deficit of GH and insulin-like growth factor-I (somatotropin C) (IGF-I/SMC) in mediating the effects of propylthiouracil (PTU)-induced hypothyroidism on body and skeletal growth and myelination was studied in the neonatal rat. Myelination (as assessed by 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP) activity), skeletal growth (as assessed by tail length) and body weight of pups from PTU-treated mothers were significantly retarded compared with normal animals or euthyroid controls. At 20 days after birth, plasma GH in hypothyroid animals was undetectable (<10 µg/l), pituitary GH content was 1.2% of control, and plasma, liver and kidney IGF-I/SMC concentrations were 63, 68 and 50% of control values respectively. CNP activity in hypothyroid brain was 52% of normal controls but the concentration of IGF-I/SMC was 113–154% of control. Treatment of hypothyroid animals from day 1 with GH (10 mg/kg body weight per day) restored liver and plasma IGF-I/SMC concentrations at 20 days to values above those of normal animals and euthyroid controls. The concentration of IGF-I/SMC was also significantly (P<0.001) restored in hypothyroid kidney (79% of normal), but the concentration in brain was unaffected. These observations provide evidence that the GH treatment employed in the present experiments was adequate to restore the deficit. GH treatment had no significant effect on tail length or CNP activity, and only a small (4–24%) effect on body weight at 20 days. Only thyroxine was able fully to restore body weight and substantially restore tail length and CNP activity.

The present study provides strong evidence against an important involvement of GH or IGF-I/SMC in mediating the effects of thyroid hormone on myelination and body growth in the infant rat. It does not, however, rule out the possibility that thyroid hormone is required for the expression of the growth-promoting effects of IGF-I/SMC by other mechanisms such as the expression of the IGF-I/SMC receptor.


INTRODUCTION

The extent to which growth hormone (GH) mediates the effects of thyroid hormone on body growth and brain development has been widely studied in juvenile rats. However, there have been few studies in the infant rat. In particular, there is conflicting information regarding the first 3 weeks after birth, an important period of brain development and rapid growth in this species, and a period during which some evidence suggests that the hormonal requirements for growth may be different from those of older animals (Walker, Simpson, Ashling & Evans, 1950; Glasscock & Nicoll, 1981).

With regard to the effects of GH on growth in the infant rat, Geel and Timiras (1970) demonstrated only a small response at 20 days of age following treatment of rats which had been thyroidectomized on day 1. In contrast, Krawiec, Garcia Argiz, Gomez & Pasquini (1969) showed a substantial growth response at 20 days of age in rats which had been radiothryoidectomized at birth and treated with bovine GH (bGH) from day 10. On the basis that this response was greater than that obtained with tri-iodothyronine (T3), these workers concluded that the failure of growth was an indirect effect of thyroid hormone deficit acting through a lack of GH. In another study, the effect of propylthiouracil (PTU)-induced
hypothyroidism on growth and plasma concentrations of GH and insulin-like growth factor (IGF) was examined in neonatal rats (Burnstein, Drazin, Johnson & Schalch, 1979). Although decreased levels of GH and insulin-like growth factors (somatomedins) (IGF/S) were shown to accompany growth retardation in their infant rats, the effects of GH treatment were studied only in adult animals where no effect on plasma IGF/S could be demonstrated. On this basis they concluded that thyroid hormone may have a direct effect on IGF/S synthesis and/or release.

With regard to the effects of GH on myelination, a number of recent studies using cultured brain cells have reported stimulatory effects of either GH (Almazan, Honegger, Matthieu & Guentert-Lauber, 1985) or insulin-like growth factor-I (somatomedin C) (IGF-I/SMC) (Lenoir & Honegger, 1983; McMorris, Smith, DeSalvo & Furlanetto, 1986) on the activity of the myelin marker enzyme 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP) (E.C. 3.3.4.37), and one study has reported a stimulation of IGF/S production in response to added T₃ (Binoux, Faivre-Bauman, Lassarre et al. 1985). These results are consistent with a possible mediation of the effects of thyroid hormone on myelination by IGF-I/SMC. In contrast, a number of animal studies, mainly in the Snell dwarf mouse, have concluded that GH is not able to promote myelination under conditions of thyroid deficiency (De Raveglia, Ghittoni & Gomez, 1974; Noguchi, Sugisaki & Tsuchiya, 1982; Noguchi & Sugisaki, 1986), although one study has concluded otherwise (Sarlieve, Bouchon, Koehl & Nesovik, 1983).

In the absence of measurements of IGF-I/SMC in the animal studies, the possibility cannot be excluded that the inability to produce a restoration of growth or CNP activity following GH treatment may be due to ineffective treatment methods. In the present study we have re-examined the possible involvement of IGF-I/SMC in mediating the effects of thyroid hormone on growth and myelination in the infant rat, by examining the response of the hypothyroid pup to GH. Unlike other studies of this kind we have established the efficacy of our treatment methods by monitoring tissue and plasma levels of IGF-I/SMC following treatment with GH.

MATERIALS AND METHODS

Animals and treatments
Three experiments were conducted. In the first, the effects of hypothyroidism on pituitary and plasma GH and on plasma and tissue concentrations of IGF-I/SMC were studied. In the second experiment the effects of administration of rat GH (rGH) and thyroxine (T₄) on growth of hypothyroid pups during the first 20 days after birth were examined, and in the third experiment the effects of rGH and T₄ on growth were again examined, together with the response of plasma and tissue concentrations of IGF-I/SMC to these hormones, in order to assess the adequacy of the GH treatment methods.

In all experiments female hooded Wistar rats were housed in air-conditioned quarters and a pelleted stock diet was freely available. Lights were on between 06.00 h and 18.00 h. Day 0 of pregnancy was taken as the day on which sperm were detected in a vaginal smear after overnight mating. Hypothyroidism was induced by daily treatment with PTU (40 mg by stomach tube) (Sigma Chemical Co., St Louis, MO, U.S.A.) from day 12 of pregnancy until termination, as previously described (King, Smith & Dreosti, 1983). Induction of hypothyroidism relies on transfer of PTU to the fetus through the placenta and to the suckling pups through the milk. Control animals nearly always gave birth on day 22 whereas hypothyroid animals tended to give birth 1 day later. For clarity of data presentation, however, day 22 was taken as the day of birth for all animals. Litters from control animals were reduced to two or eight on the day of birth (depending on the experiment) and one male pup was randomly selected for experimental use. Litters from PTU-treated animals were reduced to three males on the day of birth and to two the next day (day 1) to ensure survival. Where appropriate, daily administration of GH (10 mg/kg body weight) (NIADDK, Baltimore, MD, U.S.A.) or l-thyroxine (10 μg/kg body weight; Sigma Chemical Co.) was commenced on day 1. One of the two pups from each litter was randomly selected to receive hormone (s.c. in the scapular region) whilst its littermate received an equivalent volume of vehicle. Rat GH (rGH-B-9) from the NIADDK, was dissolved in Na₂CO₃/NaHCO₃ (0.05 mol/l) buffer, pH 9.5, and stored for up to 1 week at −80°C. T₄ (Sigma Chemical Co.) was dissolved daily in NaOH (0.01 mol/l) and diluted in Na₂CO₃/NaHCO₃ (0.05 mol/l) buffer, pH 9.5, for injection. Injections (based on daily body weight) were made between 16.00 and 17.00 h except on the last occasion before termination when they were given between 19.00 and 19.30 h. Animals were killed 12–16 h later. This timing was based on the results of D’Ercole, Stiles & Underwood (1984) which showed that the maximum tissue response of IGF-I/SMC concentration to GH administration in hypophysectomized rats was seen 12–16 h after the last injection of hormone.

Preparation and extraction of tissues
Tissues were removed from each pup, snap-frozen in liquid nitrogen and stored at −80°C for later
analysis. In the case of the brain, the olfactory lobes were first discarded and the remaining brain bisected by a vertical cut between the posterior surface of the colliculi and the anterior edge of the cerebellum. The anterior portion was retained for analysis. Blood was collected by cardiac puncture, and plasma immediately prepared and stored at -80 °C. Samples from 8-12 fetuses from each mother were pooled in order to obtain sufficient material for analysis.

When required, pituitaries for GH assay were thawed and immediately homogenized in NaOH (0.01 mol/l). The resulting homogenates were diluted immediately in NaPO4 (0.01 mol/l) buffer, pH 7-6, containing 8.8 g NaCl, 1 g NaNO3, and 1 g bovine serum albumin (BSA; Sigma radioimmunoassay grade)/l and stored at -80 °C until assay.

Samples of brain, liver and kidney for IGF-I/SMC assay were crushed under liquid nitrogen and extracts prepared in acetic acid (1 mol/l) as described by D’Ercole et al. (1984) and Vileisis & D’Ercole (1986). Extracts were immediately freeze-dried and stored at -80 °C until assay. Plasma was subjected to acid-ethanol extraction by the method of Daughaday, Mariz & Blethen (1980) to remove binding protein.

**Assays for GH**

Pituitary extracts and plasma were assayed for GH by a double-antibody method using a kit supplied by the NIADDK (lot rGH-I-4 for iodination, lot rGH-RP-1 for reference preparation and antibody anti-rGH-S-4). The hormone was iodinated with Na125I (Amersham International plc, Bucks, U.K.) in NaPO4 (0.5 mol/l), pH 7-6, by the ‘iodogen’ method (Pierce Chemical Company, Rockford, IL, U.S.A.). 125I-labelled rGH was separated from free iodine on a DEAE A-25 Sephadex column and the peak fraction of radioactivity stored at -80 °C in buffer (0.04 mol NaPO4/l, 0.01 mol EDTA/l, pH 7-6, containing 2.5 g BSA/l) and used within 1 week. Each assay tube contained 0.05 ml sample (plasma or pituitary extract), 0.25 ml assay buffer A (0.01 mol NaPO4/l, 0.025 mol EDTA/l, pH 7-6, containing 8.8 g NaCl, 1 g NaN3, and 1 g BSA/l), 0.1 ml 125I-labelled rGH in assay buffer A and 0.1 ml antibody (1:6000 dilution) in assay buffer A. After incubation at 4 °C for 18 h the following were added at room temperature: carrier monkey serum (final dilution 1:880, Commonwealth Serum Laboratories, Parksville, Victoria, Australia), goat anti-monkey antiserum (final dilution 1:120, Antibodies Inc, Davis, CA, U.S.A.) and polyethylene glycol 6000 (final concentration 3%). After 30 min the tubes were centrifuged, the supernatant aspirated and the radioactivity in the pellet measured in an LKB model 1272 γ-counter at an efficiency of approximately 80%.

To exclude the influence of interassay variability, all samples for each tissue from each experiment were included in the same assay. For plasma assays, an equal volume of plasma from hypophysectomized adult rats was included in the standards.

**Assays for IGF-I/SMC**

Duplicate samples of plasma and tissue extracts were used for measurement of IGF-I/SMC by a modification of the radioimmunoassay of Furlanetto, Underwood, Van Wyk & D’Ercole (1977) as previously described (Lineham, Smith, Dahlberg et al. 1986). 125I-Labelled IGF-I/SMC was obtained from Nichols Institute Diagnostics (San Juan Capistrano, CA, U.S.A.), and rabbit antiserum to IGF-I/SMC was provided by the NIADDK. Plasma extracts were assayed at a final dilution of 1:200 in an assay volume of 0.5 ml. Freeze-dried tissue extracts were reconstituted in Tris-HCl (0.05 mol/l), pH 7-8, in a ratio of 2 ml buffer/g original tissue and briefly centrifuged to remove insoluble material. Extracts from brain were not diluted for assay and extracts from liver and kidney were used at dilutions of 1/10 and 1/40 respectively. Each assay tube contained 0.05 ml extract, 0.25 ml assay buffer B (0.03 mol NaPO4/l, 0.01 mol EDTA/l, pH 7-5, containing 2 g NaN3 and 2.5 g BSA/l), 0.1 ml tracer in assay buffer B and 0.1 ml antibody (1:2200 dilution). Following incubation at 4 °C for 16-18 h, bound and free ligand were separated by second antibody precipitation, using sheep anti-rabbit gamma globulin (Silenus Laboratories, Dandenong, Victoria, Australia) and normal rabbit serum carrier (Commonwealth Serum Laboratories). Ice-cold buffer (1 ml) was added, the tubes centrifuged and the supernatant aspirated. Radioactivity was measured in the pellets. All measurements of IGF-I/SMC were made relative to a pool of adult human plasma which was assigned a value of 1 unit/ml. For tissue extracts, the composition of the final assay medium was adjusted, with regard to all components, to that which resulted when neutralized acid-ethanol extracts of plasma were assayed. For each experiment, all samples of each tissue or plasma were assayed together to avoid the influence of interassay variability. No attempt was made to demonstrate parallel displacement curves for rat plasma and tissue extracts compared with the human plasma extract reference.

Note that no correction was made for the contribution of IGF-I/SMC in blood trapped in tissues. Using the data of D’Ercole et al. (1984) for the blood content of adult rat liver and brain and the present values for IGF-I/SMC concentration in plasma (Table 1), only approximately 0.007-0.014 units
IGF-I/SMC per g liver and 0.002–0.003 units IGF-I/SMC per g brain could be accounted for in this way.

Assays for CNP

Portions of the same crushed brain samples from experiment three that were used for extraction of IGF-I/SMC were also used for measurement of CNP activity. Homogenates were prepared in ice-cold distilled water and treated with sodium deoxycholate as described by Kurihara & Takahashi (1979). Enzyme activity was measured by their colorimetric method using adenosine 2',3'-monophosphate as substrate (Kurihara & Takahashi, 1979).

RESULTS

Effects of hypothyroidism on tissue and plasma IGF-I/SMC

The effects of maternal PTU treatment on pituitary GH content and plasma GH concentration in fetal and neonatal rats are shown in Fig. 1. Pituitary GH content was markedly reduced in hypothyroid animals although this was not evident at 19 and 21 days of gestation. At 19 days of gestation, the pituitary GH content of hypothyroid fetuses was 53% of control values and at 20 days after birth this had fallen to 1.2% of control (Fig. 1a). Except at 6 days after birth, the effect of hypothyroidism on plasma concentrations of GH (Fig. 1b) generally reflected the effect on pituitary GH content, with a significant (*P<0.01, Student's t-test) reduction at 21 days post-conception and extremely low values at 20 days after birth. It should be noted that the value for control pups at 6 days after birth is the mean of only two animals and so should be interpreted with caution.

Concentrations of IGF-I/SMC in liver, brain and plasma of hypothyroid and control animals in experiment 1 are shown in Fig. 2. In control animals, liver IGF-I/SMC concentration increased almost sixfold between 19 days of gestation and 20 days after birth. The IGF-I/SMC concentration also increased in hypothyroid liver, but at a much lower rate so that levels were significantly lower than in controls at all postnatal ages.

The plasma concentration of IGF-I/SMC appeared to show a different pattern of development from that of liver IGF-I/SMC between 19 days of gestation and 6 days after birth. Thus whereas the measured IGF-I/SMC concentration in plasma decreased by approximately 20% in normal animals during this time, the concentration in liver increased almost threefold. However, it must be recognized that the results obtained for fetal plasma in the present study may be influenced by possible interference in the radioimmunoassay for IGF-I/SMC by the high levels of multiplication stimulating activity (rIGF-II) (MSA/IGF-II) reported to be present in fetal rat serum (Moses, Nissley, Short et al. 1980). In contrast to liver, IGF-I/SMC in brain (Fig. 2c) tended to

decrease between 6 and 20 days after birth in control animals, whereas the opposite was the case in hypothyroid animals so that at 20 days the concentration of IGF-I/SMC in hypothyroid brain was significantly (P<0.05) higher than in controls.

The tendency for increased IGF-I/SMC concentrations in hypothyroid brain at 20 days was confirmed in the third experiment (see Table 1), although the difference did not reach statistical significance.

**Effects of hypothyroidism and hormone treatment on growth**

The effects of treatment of hypothyroid pups from day 1 with rGH or T_4 on body weight (experiment 2) are shown in Fig. 3. In this experiment control litters were culled to either two or eight pups, but since there was no significant effect of litter size on the growth pattern of the pups, the data were combined for Fig. 3 (body weight at 20 days, 40.4±0.6 g (s.e.m.) (n=6) for litters of two, 39.0±1.4 (n=7) for litters of eight). Similarly there was no significant difference between the growth patterns of the untreated hypothyroid littermates of rGH- and thyroxine-treated pups, and these data were therefore also combined.
As expected, treatment of mothers with PTU retarded body growth of pups both in terms of body weight and tail length. Administration of \( T_4 \) (10 \( \mu \)g/kg body weight per day) to the pups restored body weight to normal by day 14. Tail length, which can be used as an index of skeletal growth, was also substantially restored towards normal (hypothyroid, 47±0.9 mm \((n=13)\); \( T_4 \)-treated, 65±1.8 mm \((n=6)\); normal controls, 71±0.9 \((n=13)\) at 20 days). In contrast to the effects of thyroxine, rGH (10 mg/kg body weight per day) had only a small although significant \((P<0.05)\) effect on body weight of hypothyroid pups at 20 days. The body weight of rGH-treated animals, however, remained significantly \((P<0.01)\) less than either \( T_4 \)-treated or normal controls at 20 days. Tail length was not significantly increased \((50±1.2 \text{ mm} \,(n=7))\).

In the final experiment, the effect of hormone treatment on body weight and tail length was again examined and, in addition, IGF-I/SMC concentrations were measured in plasma, liver, kidney and brain to monitor the efficacy of hormone treatment. In this experiment, whereas treatment with \( T_4 \) again restored body weight (control, 39.8±1.4 g; \( T_4 \)-treated, 37.2±1.8 g), rGH treatment resulted in only a small \((4\%)\) and statistically insignificant increase at 20 days, and rGH treatment again had no significant effect on tail length (data not shown). The reason for the difference in response of body weight to rGH between the two experiments is not immediately obvious, but may relate to differences in the degree of thyroid deficiency induced in the two experiments (see Discussion). Reference to Table 1 shows that treatment of hypothyroid pups with rGH was effective in restoring plasma and liver IGF-I/SMC concentrations to super-normal values and kidney IGF-I/SMC concentrations to close to normal values, but had no effect on the concentration of IGF-I/SMC in brain at 20 days of age. Treatment with \( T_4 \) also restored IGF-I/SMC concentrations to normal or close to normal values in plasma and kidney, but not in liver. Brain concentrations were not significantly affected. The apparent ability of thyroid hormone to restore plasma levels of IGF-I/SMC in the absence of restoration of liver concentrations is somewhat surprising in view of the fact that the liver is an important source of the plasma peptide. An explanation is not obvious, although it must be realized that a low concentration of the peptide in liver does not necessarily imply a low rate of synthesis and secretion.

### DISCUSSION

#### Effects of hypothyroidism and GH replacement on body growth

The present work confirms earlier studies which have shown reduced concentrations of plasma or pituitary GH (Mosier, Dearden, Jansons & Hill, 1977; Burnstein et al. 1979; Corio, Braverman, Christianson et al. 1979; Walker & Dussault, 1980) and plasma IGF-I/SMC (Burnstein et al. 1979; Chernausek, Underwood & Van Wyk, 1982) accompanying growth retardation in hypothyroid rats. The present study extends these observations to show that the concentration of IGF-I/SMC in the kidney and liver, a major source of IGF-I/SMC, is also markedly reduced in the neonatal hypothyroid rat.

There is now strong evidence that the growth-promoting effects of GH are brought about through the binding of the hormone to its cell surface receptor, followed by production of IGF-I/SMC (Schoenle, Zapf, Hauri et al. 1985). The ability of our hypothyroid rats to respond to GH administration with restoration of plasma, liver and kidney IGF-I/SMC...
levels indicates that GH receptors which develop during the neonatal period (Maes, De Hertogh, Watrin-Granger & Ketelslegers, 1983) remain functional in these animals, and that the dose of GH employed was adequate to replace the hypothyroid deficit. The lack of restoration of growth is, therefore, clearly not due to a defect at the level of the GH receptor. This is consistent with another study in juvenile rats which showed no depletion of GH receptors in the livers of thyroidectomized animals and the restoration of plasma IGF-I/SMC levels towards normal values following treatment with human GH (Chernausek et al. 1982). In contrast, Burnstein et al. (1979) using a protein-binding assay which also bound IGF-II/MSA, were unable to show any restoration of plasma IGF/SM in adult hypothyroid rats treated with bGH for 7 days. The reason for this difference is not apparent. It is unlikely to relate to hormone dose as Burnstein et al. (1979) employed a dose approximately 2.5 times that used by Chernausek et al. (1982). It is possible that it may relate to the nature of the assays for IGF/SM employed or to the age of the animals since there is evidence that the hormonal requirements for growth of infant rats may be different from those of older animals (Walker et al. 1950; Glasscock & Nicoll, 1981).

In the present study, rGH treatment led to the restoration of plasma IGF-I/SMC levels in hypothyroid animals to values higher than those of T₄-treated or normal animals but this was not accompanied by any significant increase in skeletal growth as assessed by tail length, and had only a small (4–24%) effect on body weight. In addition, GH-treated pups retained their cretinoid characteristics including delayed eye opening, foreshortened snout and poorly developed ears, as reported by others (Scow, Simpson, Asling et al. 1949; Geel & Timiras, 1970). Treatment with T₄ had marginally less effect than treatment with GH on plasma and tissue IGF-I/SMC levels but, in contrast, body weight was fully restored and skeletal growth substantially restored. This eliminates the possibility that other factors, such as limitation of milk supply from the hypothyroid mother, played a significant role in producing the growth retardation of hypothyroid pups.

Taken together, these results represent strong evidence against an important direct involvement of the GH deficit seen in hypothyroidism in mediating the effects on body and skeletal growth in the infant rat.

A number of other studies have examined the effects of GH administration on body growth of rats which had been thyroidectomized at birth. Only two have been concerned with the early postnatal period when hormonal requirements for growth may be different from those of the juvenile and later periods (Walker et al. 1950; Glasscock & Nicoll, 1981). The results from those studies have, however, been conflicting. Thus although Salmon (1941) was unable to show any response of body weight to GH treatment from about 4 weeks of age, others have shown a substantial response under similar conditions (Scow & Marx, 1945; Scow et al. 1949; Ray, Simpson, Li et al. 1950). In studies like the present ones using younger animals, Geel & Timiras (1970) showed only a small effect on body weight at 20 days following GH treatment from day 6, but Krawiec et al. (1969) showed a substantial response following treatment only between days 10 and 19. These latter results are puzzling since, even though the dose of GH employed in that study was substantially less than that used in the present work and by Geel & Timiras (1970), the growth response with GH was greater than that obtained with thyroid hormone.

Although the present study does not address the question, other evidence suggests that thyroid hormone may be necessary for the full expression of the growth-promoting effects of GH (Salmon, 1941; Scow & Marx, 1945; Scow et al. 1949; Burch & Van Wyk, 1987), and so it is possible that the differences in response of body growth to GH noted above may relate to the degree of thyroid deficiency induced in the respective experiments. In particular, some early animal studies showed that the growth response to GH of young rats which had retained a thyroid fragment following attempted thyroidectomy at birth, was greater than the response of those animals which were shown to be totally thyroidectomized (Salmon, 1941; Scow & Marx, 1945). Consistent with this, the dose of GH required to produce a given weight gain in young thyroidectomized rats has been reported to be 20–25 times that required to produce the same weight gain in hypophysectomized animals of about the same age (Scow et al. 1949).

Tissue culture studies have shown that the growth response of chick cartilage to IGF-I/SMC (Burch & Van Wyk, 1987) and the binding of an IGF fraction by arterial smooth muscle cells (Pfiefe & Ditschuneit, 1983) is dependent on added T₄, suggesting that thyroid hormone control of the response to GH may be exerted at the level of the IGF-I/SMC receptor. Thus in the present study, it is possible that GH was unable to bring about a full restoration of growth because of a deficiency of IGF-I/SMC receptors.

**Effect of hypothyroidism and GH on IGF-I/SMC and CNP activity in brain**

A number of recent studies have demonstrated effects in brain cell cultures which are consistent with mediation by IGF-I/SMC of the effects of thyroid hormone on myelination. First, T₄ at certain concentrations seems to increase the level of IGF/SM in cultures of
fetal mouse hypothalamus cells, presumably by a direct mechanism that does not involve GH. Secondly, other studies have demonstrated effects of GH (Almazan et al. 1985) or IGF-I/SMC (Lenoir & Honegger, 1983; McMorris et al. 1986) on the myelin-marker enzyme CNP. The effects of IGF-I/SMC have been most marked and it is possible that the effects reported by Almazan et al. (1985) were due to IGF-I/SMC produced by the brain cells in response to added GH.

The present study, however, provides no evidence to support a mediation of the effects of thyroid hormone by IGF-I/SMC in the neonatal hypothyroid rat forebrain. Thus IGF-I/SMC concentrations in forebrain at 6 and 13 days of age were not significantly affected by hypothyroidism (Fig. 3c), and at 20 days tended to be higher than in controls (Fig. 3c and Table 1). We are not aware of any other studies which have examined the effects of hypothyroidism on IGF-I/SMC in brain, although the effects of hypophysectomy have been reported for the adult rat. Consistent with the present study, no effect was shown in brain despite reduced levels in all other tissues examined (D’Ercole et al. 1984).

Although the absence of any reduction of IGF-I/SMC concentration in hypothyroid brain suggested that the peptide did not mediate the effects of thyroid hormone on myelination, we examined the effects of GH administration on CNP activity in hypothyroid animals in order to provide more direct evidence. As expected, treatment of hypothyroid pups with thyroid hormone restored activity to close to normal values at 20 days (see Table 1). Treatment with GH, on the other hand, had no effect at all, even though plasma IGF-I/SMC levels in these animals were restored to values above those seen in T$_3$-treated and normal controls (see Table 1). Although one study has produced results to the contrary (Sarlieve et al. 1983), the lack of effect of GH in the present study is in agreement with a number of previous reports which have examined the response of various indices of myelination in hypothyroid animals (De Raveglia et al. 1974; Noguchi & Sugisaki, 1986).

The differences between the in-vivo and in-vitro findings are intriguing. It should be noted that anions of thyroid hormone on IGF/SM in vitro were relatively small and variable. In addition, the precise nature of the IGF-SM was not identified, and, at higher concentrations, T$_3$ led to decreased levels of IGF/SM. In the absence of measurements of free T$_3$ levels in brain in the present study, however, it is difficult to suggest a reason for this difference. With regard to the effects of GH and IGF-I/SMC, it may be relevant that systemically administered GH is unable to enter the brain of the rat (Mayberry, Van den Brande, Van Wyk & Waddell, 1971) and so would not itself be expected to influence brain processes in vivo. It has been suggested that GH procures any effects in the brain through the production of IGF/SM in other tissues (Almazan et al. 1985). The presence of IGF transporter systems in brain (Pardridge, 1986) is consistent with this suggestion. Notwithstanding this, restoration of circulating IGF-I/SMC levels by GH in hypothyroid rats had no effect on CNP activity in brain. It may be significant that the in-vitro studies with IGF-I/SMC (Lenoir & Honegger, 1983; McMorris et al. 1986) were conducted in the presence of T$_3$, whereas the present results were obtained under conditions of low circulating thyroid hormone concentration. The differences between the in-vitro and in-vivo studies are again consistent with a regulation of IGF-I/SMC receptors by thyroid hormone as discussed earlier, with respect to the studies on body growth. Certainly the effects of GH on myelin basic protein in brain cell cultures appear to be totally dependent on the presence of T$_3$ (Almazan et al. 1985).

In summary, the present work confirms those limited previous observations which have shown only a small response of body weight and no significant response of myelination following GH administration in the hypothyroid infant rat. Unlike previous work, the adequacy of GH treatment to restore circulating IGF-I/SMC levels has been demonstrated so providing strong reasons for acceptance of the negative findings.

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REFERENCES


