Induction of reversible growth retardation and growth hormone deficiency by blockade of norepinephrine synthesis in the rat*

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Norepinephrine is a major regulator of the release of growth hormone. Diethyldithiocarbamate, a dopamine-β-hydroxylase inhibitor, reduces norepinephrine synthesis and acutely inhibits growth hormone (GH) secretion. To investigate the long-term effects of dopamine-β-hydroxylase blockade on growth, we administered diethyldithiocarbamate (0, 40, 100 or 400 mg/kg sc b.i.d.) to 21-day-old female rats for 10 days. Food intake, body weight, and tail length were measured twice a week. Plasma GH levels and hypothalamic dopamine and norepinephrine content were measured; messenger ribonucleic acids (mRNAs) for GH-releasing hormone and somatostatin were determined by quantitative in situ hybridization. Diethyldithiocarbamate administration decreased GH levels (p<0.05) and retarded growth in a dose-dependent manner (p<0.05), without altering food intake. Co-administration of GH partially reversed the growth retardation in diethyldithiocarbamate-treated animals (p<0.05). Diethyldithiocarbamate treatment also increased the hypothalamic dopamine/norepinephrine ratio (1.13 vs 0.41 control, p<0.05). Local levels of GH-releasing hormone and somatostatin mRNA were not altered by treatment. After discontinuation of diethyldithiocarbamate, growth rates returned to normal or transiently even to supranormal values. Norepinephrine synthesis blockade with diethyldithiocarbamate provides a model for reversible growth retardation, in which GH levels are decreased in the absence of decreased GH-releasing hormone mRNA. These results support a role for norepinephrine in the regulation of normal growth.

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Growth hormone deficiency (GHD) is an important endocrine cause of short stature and can occur as a permanent or transient condition (1). After reversal of GHD or GH-resistant states, such as malnutrition, subjects resume normal growth velocity but the extent of accelerated "catch-up" growth varies.

Three rodent models of GHD have been developed: hypophysectomy (2), neonatal administration of monosodium glutamate (MSG) (3–6) and the inbred dwarf mouse (7, 8). Hypophysectomy produces many other hormonal deficiencies besides GHD. Administration of toxic amino acids such as MSG results in abnormal behavior, such as tail self-mutilation; and there may be other hormonal abnormalities besides GHD. Colonies of inbred strains are difficult to obtain and to breed, and hormonal or metabolic alterations other than GHD also may cause growth retardation. All of these models have irreversible GHD.

Norepinephrine (NE) is an important regulator of GH secretion in the brain (9, 10). Exogenous administration of NE or other α-adrenergic agonists evokes GH secretion (11, 12). Blockade of NE synthesis using the dopamine-β-hydroxylase (DBH) inhibitor diethyldithiocarbamate (DDTC) (13, 14) has been studied widely as a means of acutely suppressing GH secretion. As DDTC administration results in a rapid decrease in spontaneous (11) and GHRH-induced GH secretion in rats (15) and in monkeys (16), GH release appears to be dependent on endogenous NE.

The effects of chronic DDTC administration on GH and growth have not been defined. We hypothesized that chronic DDTC administration would reduce GH levels and therefore retard growth. We anticipated that the effects of DDTC would be reversible, with recovery of GH secretion and growth following discontinuation of the drug.

To investigate this hypothesis, we treated juvenile female rats with DDTC for 10 days and evaluated their growth and hormonal status. We also measured hypothalamic levels of dopamine (DA) and NE, and mRNAs
for GHRH and somatostatin (SRIF) to provide information on the actions of DDTC, because GHRH and SRIF interact in stimulating or inhibiting GH release. To verify that growth delay was related to GH deficiency rather than to anorexia or resistance to GH action, we monitored food intake and included a group of animals that received GH replacement therapy during treatment with DDTC.

Materials and methods

Animal protocol

The animal protocol was approved by the NICHD Animal Care and Use Committee. Animals were housed in groups in cages with free access to food and water. Food intake and weight were measured twice a week. Food intake was estimated by determining the difference between the weight of the food provided and the amount remaining at the end of the time interval. This difference was divided by the number of days elapsed and by the number of animals in each cage, to give the average daily consumption per animal. Tail length was measured from the anus to the tip of the tail.

Experiment 1. Twenty-one-day-old female rats were divided into matched groups of 10 animals and received saline or DDTC (40, 100 or 400 mg/kg, or 400 mg/kg b.i.d. plus 50 μg of recombinant human GH per day; Serono, Randolph, MA) sc b.i.d. for 10 days. Growth hormone was administered in the morning at a separate injection site.

At the end of the DDTC treatment period (31 days of life), animals were sacrificed by decapitation 2 h after the last injection. At sacrifice, trunk blood was collected for GH determination. Brains were removed rapidly and placed in liquid nitrogen for later measurements of catecholamine content (control and 400 mg/kg DDTC groups) or for in situ hybridization experiments.

Experiment 2. A separate series of four experiments assessed “catch up” growth following discontinuation of DDTC. Twenty-one-day-old animals were divided into matched groups of 20 animals receiving either saline or DDTC, 400 mg/kg b.i.d., again for 10 days. Animals were weighed and measured as described above. At 31 days, treatment was discontinued. Weight gain and tail length was assessed twice a week until weight was similar in both groups or the animals reached the age of 49 days.

Assays

Growth hormone was measured by RIA as described previously (18). Catecholamine content was determined in hypothalamic tissue dissected from the posterior margin of the optic chiasm to the anterior margin of the mammillary bodies and laterally between the hypothalamic sulci. The dorsal cut was about 3 mm from the ventral surface. Tissues were weighed and results were corrected for tissue wet weight. Tissues were homogenized in 0.3 mol/l perchloric acid and centrifuged. The supernatant was partially purified by adsorption on alumina, and catecholamines in the eluent were quantified using reverse-phase liquid chromatography with electrochemical detection, as described in Ref. 19.

In situ hybridization

Brains were sectioned with a cryostat. Coronal sections (15 μm thick) were made through the midpoint of the hypothalamic arcuate nucleus and through the region of the hypothalamic periventricular nucleus. Oligonucleotide probes for GHRH (48 bp) and SRIF (15 bp) were labeled with [35S]dATP and hybridized to sections of the arcuate and periventricular nuclei, respectively, placed against film and analyzed by quantitative autoradiography. The specificity of these probes has been described previously (20, 21). Autoradiographic film images of brain sections and 35S-labeled standards were digitized on a Macintosh II computer-based image analysis system with IMAGE software (Wayne Rasband, Research Services Branch, National Institute of Mental Health). A third-order polynomial calibration curve was constructed using the transmittance values of film images of 35S-labeled brain paste standards containing known amounts of radioactivity. Transmittance measurements for each probe were made in four to six consecutive sections from each brain region per rat, and were converted to dpm using the calibration curve. The average value for each animal in experimental or control groups was used to calculate the means (N=6 per group).

Statistical analysis

Data are shown as the means ± SEM. Catecholamine content differences were analyzed by the Student’s t-test. Differences in weights, tail lengths and GH levels and between brain regions in control and experimental groups were determined by one-way analysis of variance followed by Fisher’s probable least significant difference test and Scheffe’s F test.

Results

Growth curves for the different groups of animals in experiment 1 are shown in Fig. 1. Diethylthiocarbamate administration resulted in a dose-dependent growth retardation. At a dose of 40 mg/kg b.i.d., growth of the DDTC-treated animals was similar to controls; weights at the end of the treatment period were 99 ± 1.8 vs 99 ± 1.4 g, NS (Fig. 1 and Table 1). Diethylthiocarbamate doses of 100 and 400 mg/kg significantly reduced growth (93 ± 1.0 and 88 ± 1.0 g; p<0.005 and p<0.001, respectively). Similarly, tail length was
reduced by DDTC treatment (Table 1). Food intake was similar in control and experimental groups (6.1 g/day in control group and 6.4, 6.4 and 5.9 in the 40, 100 and 400 mg/kg DDTC-treated groups, respectively); because these are group estimates they are not subject to statistical analysis.

Growth hormone levels were reduced in the DDTC-treated rats when compared with control animals (Table 1): this effect was more marked with the 400 mg/kg than with the 100 mg/kg DDTC-treated group.

Growth hormone replacement significantly increased the growth rates, although the animals remained smaller than controls.

In the 400 mg/kg DDTC-treated group, DA levels were significantly higher when compared to controls (648 ± 187 vs 320 ± 74 μg/kg; p < 0.02). There was a trend towards reduced NE levels, although it did not reach statistical significance (588 ± 61 vs 767 ± 61 μg/kg; NS). However, the DA/NE ratio in hypothalamic tissue of DDTC-treated animals was increased significantly when compared with saline-treated animals (1.13 ± 0.23 vs 0.41 ± 0.08; p < 0.05).

Binding of the [135S]GHRH and SRIF mRNAs was highly specific for the arcuate and the periventricular nuclei, respectively. A weaker signal of SRIF hybridization also was seen in the arcuate nucleus. In the regions examined, GHRH and SRIF mRNA levels were not affected by DDTC therapy or by GH replacement (Table 1).

In experiment 2, after discontinuation of DDTC, growth rates returned to normal (Fig. 2). In three of these four studies, (Fig. 2A–C), growth of the DDTC-treated animals was accelerated following discontinuation of the drug ("catch-up" growth), so that at the end of the trials the animals’ weights were no different from the controls (p > 0.5). In the fourth study (Fig. 2D), growth returned to the same rate as controls but the DDTC-treated animals were still smaller than controls at the end of the experiment at 49 days of age, 18 days after discontinuation of DDTC.

**Discussion**

The results show that chronic administration of DDTC, a DBH inhibitor, results in blockade of NE synthesis, a decrease of plasma GH levels and a dose-dependent growth retardation in juvenile female rats. At doses of 100 and 400 mg/kg b.i.d., DDTC reduced GH, weight and tail length as compared to controls. Growth hormone treatment partially reversed these outcomes, producing significant increases in tail length and weight in DDTC-treated animals. The hypothalamic DA content as well as the DA/NE ratio were increased in the DDTC-treated animals, consistent with the expected blockade of NE synthesis.

We have reported previously that DDTC administration induces vomiting in primates (16). Although rats do not vomit, a possible explanation for the growth retardation could be an induction of anorexia by DDTC. However, food consumption was similar among all the groups studied. In humans, malnutrition is characterized by GH elevation (22, 23). This is not the case in rats (24–26). Thus, GH levels could not be used in rats as an indicator of insufficient caloric intake. Administration of

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**Table 1. Growth, growth hormone (GH) levels and hypothalamic catecholamine and mRNA content in control, diethyldithiocarbamate (DDTC)-treated and GH-replaced rats.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DDT dose (mg/kg b.i.d.)</th>
<th>N</th>
<th>Weight, day 31 (g)</th>
<th>Tail length (cm)</th>
<th>GH levels (μg/l)</th>
<th>DA (μg/kg)</th>
<th>NE (μg/kg)</th>
<th>GHRH mRNA (dpm)</th>
<th>SRIF mRNA (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (saline)</td>
<td>10</td>
<td>99.0 ± 1.4</td>
<td>12.0 ± 0.3</td>
<td>48 ± 15</td>
<td>320 ± 74**</td>
<td>767 ± 61</td>
<td>143 ± 2</td>
<td>2754 ± 160</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>10</td>
<td>99.0 ± 1.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>93.0 ± 1.0***</td>
<td>-</td>
<td>20 ± 5**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>400</td>
<td>10</td>
<td>88.0 ± 1.0***</td>
<td>11.0 ± 0.1*</td>
<td>7 ± 1**</td>
<td>648 ± 187</td>
<td>588 ± 61</td>
<td>145 ± 5</td>
<td>2538 ± 46</td>
<td></td>
</tr>
<tr>
<td>400 + GH</td>
<td>10</td>
<td>95.0 ± 1.0</td>
<td>11.5 ± 0.1*</td>
<td>-</td>
<td>-</td>
<td>141 ± 3</td>
<td>2833 ± 106</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values are means ± sem. Significantly different from controls: *p < 0.04; **p < 0.02; ***p < 0.004.
GH to malnourished patients and rats does not produce growth, because a GH resistance seems to occur (27). However, since GH replacement partially restored weight gain and tail length in our experiments it appears that the growth retardation was at least partly due to GH deficiency.

Growth hormone did not fully restore weight gain and tail length in DDTC-treated animals. Similar results have been reported previously in hypophysectomized hormone-replaced (2) and in MSG-treated rats (28), where GH replacement does not fully restore growth. This leaves open the possibility that DDTC may produce growth retardation by other mechanisms as well as by inducing GH deficiency.

The lack of changes in GHRH and SRIF mRNA content was unexpected, given the markedly decreased GH secretion in DDTC-treated animals. However, other investigators have reported unvarying levels of releasing hormone mRNAs in other systems after diverse endocrine manipulations that alter the secretion. For example, castration does not alter the hypothalamic GnRH mRNA content (24). Similarly, Swanson and Simmonds have reported that unilateral catecholamine-depleting knife cuts did not alter the levels of mRNAs for CRH or vasopressin and other neuropeptides, while dramatic changes in neuropeptide levels themselves were present (30).

The conditions and the location examined for GHRH and SRIF mRNAs were similar to those of previous reports (31, 32). In a group of normal rats treated with the same dose of GH, we have observed a decrease in GHRH mRNA content when compared to controls (unpublished observations), indicating that methodological problems are not responsible for the lack of differences observed in our study.

It is possible, therefore, that changes in hypothalamic catecholamine content may alter GHRH secretion without affecting GHRH synthesis, or alternatively that NE deficiency may uncouple translation from transcription of GHRH.

After discontinuation of DDTC, cessation of catecholamine synthesis blockade apparently reversed GHD, resulting in growth acceleration and partial or complete "catch-up" growth. Although catch-up growth is a well-recognized phenomenon, its underlying mechanisms are not well understood. As most animal models of GHD are irreversible, there have been relatively few animal studies. Other interventions such as induced malnutrition or exogenous glucocorticoid administration have been used in the past in an attempt to understand the
basis for the catch-up phenomenon; in general, rats have tended not to recover completely after growth retardation. It is not clear to what extent these findings apply to transient GHD, since it is possible that different hormonal or nutritional manipulations could lead to different responses in the growth recovery process. In this sense the reversibility of DDTC-induced growth retardation may provide a pharmacological tool to investigate the endocrine mechanisms involved in "catch-up" growth after reversal of GHD deficiency.

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