Effects of hypo- and hyperthyroidism on pancreatic TRH-degrading activity and TRH concentrations in developing rat pancreas

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Abstract. High concentrations of thyrotrophin-releasing hormone (TRH) in the rat pancreas were detected during the first few days of life decreasing thereafter while pancreatic TRH-degrading activity (TRH-DA) absent at birth appeared on day 14 and increased to reach adult values by day 21. This period of life is also remarkable by the low level of circulating thyroid hormones. Since TRH-degrading activity may be thyroid hormone dependent it was of interest to study the effects of thyroid status fluctuations both on TRH-DA and TRH content during the neonatal period.

In this study, hypo- and hyperthyroidism were induced by 6-n-propyl-2-thiouracil (PTU) and triiodothyronine (T3) respectively. Pancreatic TRH-DA and TRH concentrations were measured at different ages from birth until day 29, in treated animals and results compared to control age-matched rats.

In hypothyroid rats, pancreatic TRH concentrations remained significantly higher after day 16 while TRH-DA was lower during the whole period studied. Following T3 treatment, pancreatic TRH concentrations decreased significantly from day 3 onwards. However, no significant changes were found for TRH-DA except a two-fold increase on day 28.

These results suggest that two different mechanisms may account for thyroid hormones action: 1) a direct effect on pancreatic TRH 2) an inductive saturable effect on TRH-DA. Furthermore a fine turner modulatory role of TRH-DA on TRH concentrations cannot be excluded.

In the pancreas, high concentrations of TRH have been detected at birth, reaching a peak on day 1 and 2, decreasing thereafter (Koivusalo & Leppäluoto 1979; Martino et al. 1980; Engler et al. 1981; Aratan-Spire et al. 1984). In adult rats lower pancreatic TRH concentrations (Morley et al. 1977; Martino et al. 1978) are associated with a pancreatic TRH-degrading activity (TRH-DA) (Koivusalo 1980; Safran et al. 1982). Compared to adult, no TRH-DA was found at birth; absent during the first week of life, it appeared on day 14 increasing thereafter and reaching adult values by day 21 (Aratan-Spire et al. 1984). Since the neonatal period is characterized by low levels of circulating thyroid hormones, it has been postulated that, as for plasma (Bauer 1976; White et al. 1976; Aratan-Spire et al. 1983) thyroid status may be responsible for the peculiar developmental pattern of pancreatic TRH-DA.

In order to investigate the role of thyroid hormones on pancreatic TRH metabolism (concentrations and degradation), in vivo PTU and T3 treatments were applied from birth until adulthood, and corresponding TRH concentrations and TRH-DA were compared to those of age-matched sham-treated rats.

Materials and Methods

Products

[2,3-3H]TRH (60 Ci/mmol) was prepared according to Pradelles et al. (1972) by J. L. Morgat and H. Levine-Pinto (Laboratoire de Biochimie, CEA, Saclay, France). Synthetic TRH (pyro Glu-His-Pro NH2) was purchased from Peninsula laboratories (San Carlos, CA); TRH-OH, pyro Glu-His and histidylproline diketopiperazine (His-Pro-DKP) from Sigma Chemical Co. (Saint-Louis, Missouri). L-triiodothyronine (T3) and

Experimental animals

Experiments were performed on newborn rats ranging from 1 to 35 days of age, all of the Sprague-Dawley strain (Charles River, St Aubin les Elbeuf, France). The animals were kept at 21°C in a 12 h light-dark cycle.

Treatment protocol

Hypothyroidism was induced in pregnant rats by administering during the last week of gestation PTU (0.05%) in drinking water made palatable with D-glucose (80 g/l) combined to a low iodine diet (2 ± 0.25 µg iodine/100 g: Dr. Triantaphillidis, Villemoisson-sur-Orge, France). Control rats received a normal diet and were also provided with D-glucose in drinking water in order to achieve a similar glucose intake. Treatment was discontinued at birth to allow lactation. Infant rats were injected daily with a long-acting solution of PTU (50 µg/rat) in 1% carboxymethylcellulose. Control pups received only 1% carboxymethylcellulose in vehicle. A group treated with PTU was replaced with T₃ to euthyroid levels to evaluate a possible direct effect of PTU. Effect of T₃ was studied by giving young rats daily ip injections of T₃ (10 µg/100 g body weight) in isotonic saline (pH: 8.5). Animals were killed 24 h after the last injection. Control groups received vehicle only. T₃ treated rats were studied beginning on day 3. PTU treated rats were tested from day 1 onwards.

Preparation of samples

Trunk blood was collected on chilled heparinized tubes and plasma immediately separated by centrifugation at 4°C. T₃ measurements were done on individual plasma samples while TSH was determined on pooled samples.

Pancreatic samples for TRH-DA studies and for endogenous TRH measurements were prepared as previously reported (Aratan-Spier et al. 1984).

Measurement and expression of TRH-degrading activity

Incubation and chromatography: 45 µM of TRH containing [l-proline 2.3-HI]TRH (initial isotope specific activity: 2.1 × 10³ cpm/pmol) was incubated with fresh pancreatic homogenate appropriately diluted with phosphate buffered saline (PBS) to obtain a solution of 400 ± 42 µg of total protein in 40 µl. The degradation of TRH was determined as previously described (Aratan-Spier et al. 1984).

The initial rate of TRH degradation expressed as pmol of TRH degraded/min was used to compare pancreatic TRH-DA of treated and non-treated rats, during development. The degrading activity was also expressed as a function of the total protein concentration determined by the Lowry-Folin method (Lowry et al. 1951). This apparent specific activity was therefore expressed as pmol of TRH degraded/min/mg protein.

Radioimmunoassays

TRH RIA was performed using an antibody provided by Dr. Oliver (Marseille, France) with modifications of a previously described method (Eskay et al. 1976). Sensitivity of the assay was 13 pg/tube and intra- and inter-assay coefficients of variation, 7 and 17.5% respectively measured with a dose of 490 pg.

T₃ radioimmunoassay was performed as reported previously (Wiersinga & Chopra 1982) using specific rat thyroid-hormone-free serum for standard curve.

Statistical analysis

1) Pancreatic TRH-DA values were compared by multiple-comparison t-tests.

2) Pancreatic TRH concentrations were expressed as pg/mg wet weight ± SEM and evaluated, after logarithmic transformation, by analysis of covariance (Finney 1971) followed by multiple comparison of means.

Results

Effect of treatment with PTU

PTU-treated animals were characterized by a lower body weight, a lower plasma T₃ and a higher TSH compared to control values. Effect of this treatment on TRH-DA and TRH concentrations is summarized in Fig. 1 and Tables 1 and 2.

During the whole period studied, TRH-DA developmental pattern was different in PTU-treated animals: expressed as initial rate of degradation (pmol/min/mg total protein) (Table 1) TRH-DA is low at birth both in sham-treated and PTU-treated animals. However, the gradual increase from day 14 onward observed in sham-treated animals is absent in hypothyroid animals in which TRH-DA remained low during the whole period studied. In PTU + T₃-treated animals, TRH-DA was not significantly different from age-matched control values (Table 1).

During the first week of life, TRH concentra-
Initial rate of TRH degradation (pmol/min) by pancreatic homogenates of PTU-treated and T3-treated rats, compared to non-treated age-matched controls. Compared to age-matched controls, values are significantly lower following PTU-treatment \((P < 0.001)\); following T3-treatment, the significant increase \((P < 0.001)\) was only observed on day 28.

Apparent specific activity of pancreatic homogenates expressed as pmol of TRH degraded/min/mg total protein \pm SEM in sham-treated, T3-treated, PTU and PTU + T3-treated age-matched animals, during development. Number of animals tested are indicated in parentheses.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sham-treated</th>
<th>T3-treated</th>
<th>PTU-treated</th>
<th>PTU + T3-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>(6.26 \pm 0.57)</td>
<td>(7.12 \pm 0.91)</td>
<td>(3.44 \pm 0.66)</td>
<td>3.44 \pm 0.66</td>
</tr>
<tr>
<td>14-16</td>
<td>(57.17 \pm 1.50)</td>
<td>(59.19 \pm 6.07)</td>
<td>(2.29 \pm 0.67)</td>
<td>2.29 \pm 0.67</td>
</tr>
<tr>
<td>21-22</td>
<td>(66.22 \pm 2.93)</td>
<td>(62.56 \pm 7.55)</td>
<td>(2.19 \pm 0.94)</td>
<td>2.19 \pm 0.94</td>
</tr>
<tr>
<td>28-29</td>
<td>(39.99 \pm 4.71)</td>
<td>(79.77 \pm 4.71)</td>
<td>(4.58 \pm 1.70)</td>
<td>4.58 \pm 1.70</td>
</tr>
<tr>
<td>35</td>
<td>(38.22 \pm 3.76)</td>
<td>(44.49 \pm 2.54)</td>
<td>(4.51 \pm 0.90)</td>
<td>44.49 \pm 2.54</td>
</tr>
</tbody>
</table>

Values corresponding to PTU-treated rats are significantly lower \((P < 0.001)\) between day 7 and day 35 compared to age-matched sham-treated animals. Following T3-treatment, highly significant increase \((P < 0.001)\) compared to age-matched sham-treated animals, was only observed on day 28–29. In euthyroid rendered animals (PTU + T3-treated), apparent specific activity was not significantly different from age matched controls.
Pancreatic TRH concentrations (pg/mg wet weight ± SEM) of sham-treated, T3 and PTU-treated age-matched animals during development. Number of animals are shown in parantheses. Treated animals were compared to controls (see Materials and Methods section).

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Sham-treated</th>
<th>T3-treated</th>
<th>PTU-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>353.8 ± 46.5</td>
<td>107.4 ± 5.8**</td>
<td>348.4 ± 43.1</td>
</tr>
<tr>
<td>5</td>
<td>252.1 ± 37.7</td>
<td>66.5 ± 14.7**</td>
<td>294.4 ± 77.7</td>
</tr>
<tr>
<td>7</td>
<td>155.3 ± 23.3</td>
<td>58.0 ± 9.8*</td>
<td>251.7 ± 45.7</td>
</tr>
<tr>
<td>16</td>
<td>37.8 ± 8.5</td>
<td>10.6 ± 1.3**</td>
<td>141.3 ± 65.3**</td>
</tr>
<tr>
<td>22</td>
<td>11.9 ± 1.4</td>
<td>2.5 ± 0.8**</td>
<td>29.9 ± 5.1*</td>
</tr>
<tr>
<td>29</td>
<td>2.0 ± 0.6</td>
<td>0.4 ± 0.1***</td>
<td>7.2 ± 1.4**</td>
</tr>
</tbody>
</table>

*: P < 0.05; **P < 0.01; ***P < 0.001.

Changes in age dependent decrease pattern of P-TRH following PTU and T3-treatment

The pancreatic TRH decrease was found to be age dependent in sham-treated rats (r = -0.971, P < 0.001), in PTU-treated rats (r = -0.930, P < 0.001) and in T3-treated rats (r = -0.967, P < 0.001).

Additionally, the comparison of the age dependent decrease patterns between sham-treated, PTU-treated and T3-treated rats studied by analysis of covariance showed non-parallelism between all three slopes (Fig. 2).

Discussion

Previous studies have substantiated the hypothesis that thyroid hormones may regulate TRH-DA: in adult hypothyroid rats a decrease in TRH-DA is observed in the plasma (Bauer 1976; White et al. 1976). During the neonatal period, TRH-DA is low both in plasma and in pancreas and increases in parallel with the elevation of plasma T4 and T3 concentrations. Following PTU-induced hypothyroidism, however, a delayed development of TRH-DA was observed both in plasma and in pancreas. The same treatment applied to adult rats leads to a significant decrease of pancreatic TRH-DA associated with a 10-fold increase of TRH concentrations (Wolf et al. 1984). These observations suggest that in this tissue TRH concentrations may be regulated by TRH-DA.

The aim of this study was to investigate the two following hypotheses: 1) pancreatic TRH-DA is regulated, as in plasma, by thyroid hormones; 2) pancreatic TRH is modulated by pancreatic TRH-DA.

Our results indicate that pancreatic TRH-DA, which appears in sham-treated rats by day 14 and increases thereafter, remains low in PTU-treated rats during the whole period considered. Simultaneous 7 day PTU + T3-treatment restores the pancreatic TRH-DA values to control levels. Thus a direct effect of PTU may be discarded.

Compared to sham-treated age-matched animals, TRH concentrations were higher following PTU-treatment, between day 7 and day 29. Responses of the neonatal pancreas to PTU-treatment were similar to those of the adult pancreas. The absence of TRH-DA offered a good model to relate fluctuations in thyroid hormones levels to changes in
TRH concentrations: in fact, no TRH-DA was found 1) in normal or treated animals during the first week of life; 2) following PTU-treatment during the whole period studied.

1) In the first week of life, TRH concentrations decreased following T₃-treatment. This suggests a direct effect of thyroid hormones levels on the mechanisms regulating TRH metabolism such as biosynthesis or/and secretion.

2) Following PTU-treatment, the severe diminution of TRH-DA did not provoke the total abolition of age dependent decrease of TRH. Nevertheless, compared to sham-treated animals, the decrease was found to be slower.

In light of these observations we postulate a fine tuner modulatory role – if any – for TRH-DA, amplifying or not the main T₃ direct effect on pancreatic TRH concentrations.

Furthermore, T₃-treatment produced significantly lower TRH concentrations between day 7 and day 29 while TRH-DA remained unchanged except on day 28–29.

These results suggest that thyroid hormones action on TRH-DA and TRH concentration may be triggered by two different mechanisms. Thus, T₃ action may have 1) an inductive and probably saturable effect on TRH-degrading enzyme(s), similar to the effect described for plasma TRH-DA (Bauer 1976) and 2) a direct effect on pancreatic TRH.

Immunoreactive pancreatic TRH is mainly localized in pancreatic beta cells (Martino et al. 1978; Kawano et al. 1983; Aratan-Spire et al., in press). However, the effect of thyroid treatment on pancreatic beta cells' hormones is poorly studied, even for insulin. Indeed, thyroxine treatment inhibited the insulin secretion from the perfused rat pancreas (Lenzen & Kücking 1982), although changes of insulin contents following this treatment have not been reported.

It remains hazardous to explain the changes observed following in vivo PTU or T₃-treatments in term of thyroid specific effect in the absence of data concerning thyroid hormones receptors in the developing rat pancreas. On the other hand, TRH metabolism may also be modulated by other thyroid-dependent features such as central catecholaminergic pathways (Lengvari et al. 1980; Engler et al. 1982; Smythe et al. 1982).

This first in vivo approach must be completed by in vitro studies (on thyroid receptors in the pancreas, islets of Langerhans in culture) to avoid thyroid hormones mediated indirect effects and to further investigate the function of TRH in the pancreas.

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References


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