Triiodothyronine Down-Regulates Thyrotropin-Releasing Hormone (TRH) Synthesis and Decreases pTRH-(160–169) and Insulin Releases from Fetal Rat Islets in Culture*

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ABSTRACT

TRH is localized with insulin in β-cells. It is synthesized as a prohormone containing five copies of TRH and seven cryptic peptides, including pro (p)-TRH-(160–169). Thyroid hormone regulates the expression of several genes encoding peptide hormones. We found that circulating T₃ concentrations were inversely correlated with TRH levels in two physiopathological situations. There are low concentrations of circulating thyroid hormone and very high concentrations of TRH and pTRH-(160–169) during development, and experimental hypothyroidism results in higher concentrations of prepro (pp)-TRH messenger RNA (mRNA) and TRH content in the adult rat pancreas than are present in the euthyroid pancreas.

TRH was originally isolated from the hypothalamus (1, 2), but it is also synthesized in the islets of Langherans and localized in insulin-containing cells (3–5). The TRH prohormone contains five copies of the TRH progenitor sequence, Gln-His-Pro Gly, linked by connecting peptides (6, 7). One such connecting peptide, pro (p)-TRH-(160–169), is reported to be biologically active (8, 9) and has been detected in the islets, but its secretory pattern is unknown (10). Unlike major islet hormones, however, the highest concentrations of TRH and pTRH-(160–169) are detected during the early development of neonatal rats (2–3 days after birth) (11). This period coincides with a marked growth of the β-cell population (12). This suggests that TRH is involved in the regulation of growth or fetal islets in an as yet undefined way. TRH and pTRH-(160–169) are, therefore, candidate phenotypic markers for monitoring the growth characteristics of islet β-cells. In man, the pancreatic TRH concentrations are highest between 6–8 weeks gestation, and they peak before insulin levels (13). Thus, this study may also be relevant to human islet development.

Hypothalamic TRH stimulates TSH secretion (1, 2). Pancreatic TRH is involved in the stimulation of glucagon secretion and the inhibition of exocrine pancreatic secretion (14, 15). However, despite its biological contribution as a regulatory peptide in the adult pancreas, the physiological significance of TRH in islet development remains an open question. Hence, a clear picture of the hormonal control of TRH gene expression may shed light on this point. We previously studied the regulation of islet TRH gene expression in vivo after chemical thyroidectomy. Experimentally induced hypothyroidism was associated with an increase in TRH messenger and peptide in rat islets (16–18).

Fetal islet cultures (19) provide the only available model for investigating the regulation of islet TRH gene expression and the possible impact of this regulation on islet development. We have therefore investigated the direct effect of T₃ on islet ppTRH messenger RNA (mRNA) concentrations, the TRH and pTRH-(160–169) contents and secretions, together with the concomitant regulation of insulin synthesis and secretion.

Materials and Methods

Preparation of islets

Fetal islets were prepared as described by Hellerström et al. (19). Briefly, fetuses were removed from pregnant Wistar rats at 21 days gestation. Day 0 was defined as the day on which mating occurred. Fetal pancreases were removed aseptically, placed in cold HBSS supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin, and minced. HBSS (4 ml) containing 6 mg/ml collagenase CLS 4 (Worthington Biochemical Corp., Freehold, NJ) was added to each of 4 centrifuge tubes, each containing 10–12 pancreases. The tubes were incubated in a shaking water bath at 37°C for 8 min. The resulting digested tissue was washed 3 times with cold HBSS, and the pellets were pooled and suspended in 500 μl HBSS. Aliquots of this suspension (100 μl) were placed in 50-mm plastic culture dishes and cultured for 5 days in 5 ml RPMI 1640 medium containing 11 mM glucose, 10% heat-inactivated cortisol.
FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. The culture dishes were kept at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. The growth medium was replaced every day. The islets attached to the bottom of the culture dishes were then gently blown free with a sterilized Pasteur pipette under a stereomicroscope. The detached islets were cultured free floating in 50-mm petri dishes, which did not permit cell attachment (Falcon 1007, Falcon Plastics, Los Angeles, CA), in complete RPMI 1640 medium supplemented with 10% FCS that was changed every other day. The term culture refers to the free floating islets in this study.

Chronic exposure and cumulative release experiments. The islets were collected, washed with PBS, distributed in dishes (100 islets/dish), and cultured in RPMI medium supplemented with charcoal-stripped FCS (ch-FCS; 1% charcoal for 24 h at 4°C) and 1 mM bacitracin, with increasing concentrations of T₃ for 48 h in a humidified atmosphere of 5% CO₂-95% air.

Short term release experiments. The islets were collected and washed three times with PBS, and batches of 10 islets were incubated for 3 h at 37°C with various concentrations of T₃ in 1 ml HBSS supplemented with 0.1% BSA, 1 mM bacitracin, and 2.8 or 16.7 mM glucose.

At the end of chronic and short term release experiments, the islets and media were separated and treated for peptide or RNA determinations. One group of 1500 neonatal islets were recovered from 10–15 pancreases. The number of islets per batch was routinely determined with various concentrations of T₃ in 1 ml HBSS supplemented with 0.1% BSA, 1 mM bacitracin, and 2.8 or 16.7 mM glucose.

Islet extraction for TRH, pTRH-(160–169), and insulin determinations

After chronic or short term exposure to T₃ (48 or 3 h), an inhibitor cocktail (phenylmethylsulfonylfluoride, 2-iodoacetamide, and EDTA; 1 mM each) was added, and the medium was removed. The islets were disrupted by sonication. The supernatant from the sonicated islets and the medium were then separately acidified with 1.7 m acetic acid, lyophilized, and kept for TRH, pTRH-(160–169), and insulin RIAs.

TRH was measured, using a specific antiserum, 4-B-18 (10), and [125I]TRH (2200 Ci/mmol; New England Nuclear Corp., Boston, MA). The sensitivity of the assay was 5 fmol/tube, and the intra- and interassay coefficients of variation were 3.5% and 7%, respectively. Insulin was measured using an antibody directed against a mixture of porcine and bovine insulin, of variation were 3.5% and 7%, respectively. Insulin was measured using a specific antiserum, 4-B-18 (10), and [125I]TRH (2200 Ci/mmol; New England Nuclear Corp., Boston, MA). The sensitivity of the assay was 5 fmol/tube, and the intra- and interassay coefficients of variation were 4% and 6%, respectively. pTRH-(160–169) and insulins were measured using a specific antiserum, 4-B-18 (10), and [125I]pTRH (2200 Ci/mmol; New England Nuclear Corp., Boston, MA). The sensitivity of the assay was 15 pg/tube, and the coefficient of variation within and between assays was 10%. Results are expressed as femtomoles per islet for TRH and pTRH-(160–169) and nanograms per islet for insulin.

Extraction of islet RNA

Total RNA was extracted from islets by a single step method (20). The islets were washed twice with PBS, suspended in guanidine thiocyanate-phenol-chloroform (50:25:25, vol/ vol/ vol), and precipitated twice with isopropanol. The RNA was then dissolved in distilled water. The integrity and yield of the RNA extracts were checked by absorbance at 260 and 280 nm and by electrophoresis in 1% agarose gel containing 0.01% ethidium bromide under nondenaturing conditions. The recovery of total RNA was 10 μg/400 islets.

Northern blot analysis

Samples of total RNA (4 μg) were electrophoresed under denaturing conditions in 1% agarose gels containing 2.2 m formaldehyde. The nucleic acids were vacuum blotted (Vacuum Blotter, Appligene, Strasbourg, France) to Hybond-N nylon membranes and cross-linked by UV irradiation. The membranes were hybridized with complementary DNA (cDNA) probes labeled by random priming to a specific activity of 10⁶ cpm/μg (21). Membranes were prehybridized at 65°C in 7% lauryl sulfate, 200 mM phosphate buffer (pH 7.2), 1 mM EDTA, and 1% BSA and then hybridized with the [32P]-labeled pTRH cDNA probe (10⁶ cpm/ml) for 16 h at 65°C. The membranes were then washed three times for 15 min each time at 65°C in 0.5× SSC (standard saline citrate) containing 0.1% SDS and autoradiographed using Kodak XAR 5 film (Eastman Kodak Co., Rochester, NY) at ~80°C. Blots were stripped by washing at 95°C with 0.01× SSC-0.1% SDS, 1% glycerol, and 1 mM EDTA for 30 min and were reprobed with the [32P]-labeled proinsulin (pIns) cDNA (10⁶ cpm/ml). A cDNA probe encoding 18S RNA was used to normalize the intensity of the hybridization signals and to allow for minor differences in recovery. The relative densities of the bands were determined using a computerized image analysis system, with Image 1.57 (NIH, public domain).

Probe synthesis and labeling

Propro (pp)-TRH cDNA. A 1241-bp EcoRI-PstI fragment of ppTRH cDNA inserted into the plasmid vector pSP65 was excised with EcoRI and HindIII.

pIns cDNA. A 300-bp fragment of proinsulin cDNA inserted into pUC was excised with BamHI and EcoRI (3, 22).

18S cDNA. A 1975-bp fragment of 18S cDNA inserted into pSP64 was excised with SalI and EcoRI (23).

Each insert was separated electrophoretically in a 1% low melting point agarose gel (BRL, Gaithersburg, MD), and an aliquot (60 ng) of purified insert was used as a template to prime DNA synthesis in vitro with [32P]dCTP and the Klenow fragment of DNA polymerase (Prime-a-Gene Labeling System, Promega Corp., Madison, WI).

Statistical analyses

All results are the mean ± se. The statistical significance of the differences was determined by one-way ANOVA and Student’s t test.

Results

In pilot experiments, we tested the viability, insulin and TRH contents, and secretary capacity of fetal islets grown in various conditioned media. Islets maintained for 24 or 48 h in RPMI supplemented with 10% ch-FCS had insulin and TRH contents similar to those of islets maintained under standard culture conditions. Similarly, islets maintained for 24 h in serum-free medium containing 0.1% BSA had insulin and TRH contents similar to those of islets cultured in standard medium. In contrast, insulin and TRH contents of islets incubated for 48 h in serum-free medium containing 0.1% BSA were much lower than those of islets maintained in standard culture conditions (Table 1).

The secretory capacity and content of the islets maintained in RPMI supplemented with ch-FCS were compared with those of islets grown under standard culture conditions (Fig 1). Batches of 10 islets were incubated for 3 h at 37°C in HBSS containing 2.8 or 16.7 mM glucose. The content of the islets

### TABLE 1. Insulin and TRH content of islets maintained for 24 and 48 h in RPMI containing FCS, charcoal-stripped FCS (ch-FCS), or 0.1% BSA

<table>
<thead>
<tr>
<th>Condition</th>
<th>Insulin (ng/islet)</th>
<th>TRH (fmol/islet)</th>
</tr>
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<tbody>
<tr>
<td>24 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% FCS</td>
<td>19.2 ± 1.5</td>
<td>23.1 ± 3.3</td>
</tr>
<tr>
<td>10% ch-FCS</td>
<td>20.1 ± 2.2</td>
<td>21.7 ± 1.6</td>
</tr>
<tr>
<td>0.1% BSA</td>
<td>20.1 ± 2.2</td>
<td>10.5 ± 0.3</td>
</tr>
<tr>
<td>48 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% FCS</td>
<td>15.9 ± 1.3</td>
<td>25.0 ± 3.0</td>
</tr>
<tr>
<td>10% ch-FCS</td>
<td>14.3 ± 1.1</td>
<td>20.9 ± 2.5</td>
</tr>
<tr>
<td>0.1% BSA</td>
<td>17.9 ± 3.3</td>
<td>8.2 ± 0.3</td>
</tr>
</tbody>
</table>

The preparation of islets, experimental conditions, and extraction procedures were described in Materials and Methods. Insulin and TRH contents were measured by specific RIAs. Results are the mean ± se for five to seven independent determinations.
remained unchanged. The TRH release pattern was similar to that of insulin, and both were stimulated by 16.7 mM glucose (Fig. 1). The viability of the islets maintained in medium supplemented with either ch-FCS or normal FCS were similar (data not shown). Therefore, islets treated for 48 h and used for peptide extraction were switched from standard medium to RPMI medium supplemented with ch-FCS for 2 additional days, but the intraexperiment controls were kept in standard medium. The islets were rinsed with PBS, and batches of 10 islets were incubated for 3 h at 37 C in HBSS containing 2.8 or 16.7 mM glucose, a cocktail of protease inhibitors was added, and the islets were separated from the release medium. The islets were sonicated, and sonicates and medium were acidified with 1.7 M acetic acid, lyophilized, and assayed for TRH and insulin. The contents of the islets remained unchanged. Values are the mean ± SE for 15 observations from 3 independent experiments.

**Effects of T_3 on the steady-state contents of ppTRH and pIns mRNAs in islets cultured for 2 and 7 days (Fig. 2).** Isolets cultured for 2 and 7 days (d-2 and d-7) were maintained for 24 h with or without T_3 (10^{-8} M) in RPMI medium containing 0.1% BSA containing 0.1% BSA with or without T_3 (10^{-8} M). Total islet RNA was extracted, and samples (4 μg) were loaded and size-separated on denaturing agarose gels, transferred to Hybond-N nylon membranes, and hybridized successively with random primed 32P-labeled cDNA encoding TRH, insulin, and 18S RNAs. The membranes were autoradiographed with Kodak XAR 5 film at −80 C for 48 h for ppTRH and 6 h for pIns mRNA. A, The autoradiograph shown is representative of five other blots. B, Densitometric analysis of the hybridization signals for TRH and insulin from d-2 and d-7 islet culture with (black columns) and without (white columns) T_3. The optical densities of the hybridization signals for TRH and insulin from independent experiments were averaged and corrected for 18S mRNA. The relative densities of the bands were determined by computerized image analysis (Image 1.57, NIH, public domain). Data are the mean ± SE for three to five independent experiments. **, P < 0.001 vs. control (no T_3 added).
or 10% ch-FCS. The control levels of ppTRH and pIns mRNAs and the magnitude of the decrease in ppTRH mRNA were similar regardless of the medium used or the duration of the treatment (24 or 48 h). Adding 10⁻⁸ M T₃ to d-2 and d-7 islets reduced the amounts of ppTRH mRNA to below the control values. Expressed as a percentage of the control value (100%), the magnitude of the decreases in d-2 and d-7 islets were similar: 56 ± 8% for d-2 and 48 ± 10% for d-7 islets. The amount of pIns mRNA was not affected.

**Dose-dependent effects of T₃ (Fig. 3).** The dose-dependent effects of T₃ treatment on ppTRH and pIns mRNAs were studied using d-2 islets kept for 24 h in serum-free RPMI containing 0.1% BSA. T₃ dose-dependently reduced the steady-state concentrations of ppTRH mRNA, as estimated by Northern blot analysis. Adding T₃ to the islet culture medium for 24 h produced much lower ppTRH mRNA concentrations than those in the control culture. The densitometric analysis was performed on six blots, and the results were expressed as the mean ± se. The maximal decrease was about 50% of the control value.

**Long-term T₃ treatment and the regulation of TRH, pTRH-(160–169), and insulin (Fig. 4).** The islets were randomly distributed (100 islets/dish) and maintained for 48 h in medium containing 10% ch-FCS and various concentrations of T₃. The media and islets were separately treated for peptide measurement. Each point is the mean ± se of six independent determinations.

**Changes in cumulative release.** TRH release (femtomoles per dish/48 h) was similar for all concentrations except the highest dose of T₃ (10⁻⁷ M): 61.9 ± 13.7% of the control value, for six determinations.

Adding T₃ to the islet culture medium for 48 h produced a dose-dependent decrease in cumulative pTRH-(160–169) release (femtomoles per dish/48 h). Expressed as a percentage of the control value (100%), the decrease in cumulative pTRH-(160–169) was 74.9 ± 6.4 for 10⁻¹⁰ M T₃ (P < 0.05), 60.9 ± 5.5 for 10⁻¹¹ M T₃ (P < 0.01), 50.5 ± 5.3 for 10⁻¹² M T₃ (P < 0.005), and 36.7 ± 3.7 for 10⁻¹³ M T₃ (P < 0.005). T₃ caused a smaller, but significant, decrease in cumulative insulin release (nanograms per dish/48 h). The percent decreases from the control value (100%) were 79.1 ± 3.8 for 10⁻¹⁰ M T₃ (P < 0.05), 72.9 ± 2.1 for 10⁻¹¹ M T₃ (P < 0.005), 74.6 ± 1.4 for 10⁻¹² M T₃ (P < 0.005), and 62.6 ± 3.1 for 10⁻¹³ M T₃ (P < 0.001).

**Changes in islet TRH, pTRH-(160–169), and insulin contents.** Adding T₃ to the islet culture medium for 48 h resulted in a dose-dependent decrease in TRH content. The percent decreases in TRH content from the control value (100%) were 85.3 ± 9.5 for 10⁻¹¹ M T₃, 59.1 ± 9.4 for 10⁻¹² M T₃ (P < 0.05), 54.8 ± 7.6 for 10⁻¹³ M T₃ (P < 0.05) and 41.1 ± 4.3 for 10⁻¹⁴ M T₃ (P < 0.01). The pTRH-(160–169) content was unaffected, and that of insulin was decreased to 78.4 ± 6.0% of the control value (P < 0.05) only by the highest dose (10⁻⁷ M) of T₃.

**Short-term T₃ treatment and the regulation of TRH, pTRH-(160–169), and insulin (Fig. 5).** Batches of 10 untreated islets were used to study the effect of T₃ on TRH, pTRH-(160–169), and insulin releases and contents. They were incubated for 3 h at
37°C in HBSS containing 2.8 or 16.7 mM glucose and various concentrations of T₃. The TRH, pTRH-(160–169), and insulin contents were unaffected. The molar ratio of TRH/pTRH-(160–169) in the islets was about 5. The contents and releases of the peptides were expressed as the mean ± SE of the indicated number of determinations. TRH content was 17.7 ± 2.1 fmol/islet (n = 12). The basal and glucose-induced TRH releases were 4.5 ± 0.2 (n = 5; basal) and 10.6 ± 0.5 (n = 11; glucose-induced) fmol/islet/3 h; the release/content ratios were 0.25 (basal) and 0.60 (glucose-induced) regardless of whether T₃ was present or absent.

The pTRH-(160–169) content was 3.7 ± 0.6 fmol/islet (n = 23). The basal and glucose-induced pTRH-(160–169) releases were 0.6 ± 0.1 (n = 10; basal) and 2.1 ± 0.4 (n = 10; glucose-induced) fmol/islet/3 h; the release/content ratios were 0.16 (basal) and 0.55 (glucose-induced) regardless of whether T₃ was present or absent.

Insulin content was 16.2 ± 4.5 ng/islet (n = 32). The basal and glucose-induced insulin releases were 1.60 ± 0.22 (n = 12; basal) and 4.53 ± 0.32 (n = 20; glucose-induced) ng/islet/3 h. The release/content ratios were 0.10 (basal) and 0.27 (glucose-induced).

T₃ had no acute (3 h) effect on the content or release of TRH, pTRH-(160–169), or insulin.

**Discussion**

We have examined the chronic and dose-dependent effects of T₃ on TRH and insulin gene expression in fetal islets in culture as a first step toward defining the mechanisms involved in the thyroid hormone-dependent regulation of TRH and insulin synthesis and release. These experiments were
also designed to validate in vivo observations made on hypothyroid rat pancreas (16–18) and to gain insight into the effect of T3 on islet TRH synthesis.

Fetal islets in culture are a valuable experimental model of β-cell function. The characteristics of fetal islets in culture have been extensively described. The culture of fetal cells makes it possible to prepare large numbers of islets free of exocrine tissue contamination and fibroblast-like cells (19). A histological description of these islets has been reported (20). The endocrine cells are first attached as a monolayer and then progressively reorganize into islets (25). The neofomed fetal islets are functionally immature, and so have a developing, rather than adult islet hormone secretory pattern. Their fetal character is indicated by their low sensitivity to glucose (26). Unlike intact, vascularized islets in situ, they can only undergo interstitial (paracrine-like) interactions. Studies of this primary organ culture may provide insight into islet development.

Fetal islets in culture have a higher proportion of β-cells than age-matched, neonatal islets in situ (27). Interestingly, they also have a persistent high TRH concentration throughout the culture period (28). They are, therefore, suitable for studying β-cell function. We find that T3 reduces TRH gene expression in cultured fetal islets. The decrease is similar when RNA from d-2 or d-7 islet cultures is assayed. We, therefore, performed all studies on islets cultured for 2 days to obtain the highest proportion of β-cells and to reduce the corrective effect of non-β-cells, especially on the secretory pattern of insulin (24, 26). The reduction in the steady-state concentrations of ppTRH mRNA and TRH were dose dependent, whereas the levels of pIns mRNA and insulin were unaffected by T3.

Treatment for 48 h with various doses of T3 resulted in a lower TRH content and lower amounts of cumulative pTRH-(160–169) and insulin released. To our knowledge, the secretory pattern of pancreatic pTRH-(160–169) has not been studied before, and its biological effects on islet function has not previously been reported. In agreement with a previous report, the molar ratio of TRH to pTRH-(160–169) was 5% in fetal islets (10). The molar ratio of TRH to insulin contents was about 0.5%, and that of pTRH-(160–169) to insulin was 0.1%. This study also shows that pTRH-(160–169) is secreted and that its secretory pattern is similar to that of insulin. The release of pTRH-(160–169) is glucose sensitive: 16.7 mm glucose stimulated its release, giving 3 times more release in short term experiments.

We demonstrated here that T3 down-regulates TRH gene expression in the cultured fetal islets. To increase the sensitivity of the islets to thyroid hormone, media devoid of T3 were used during the treatment. Low levels of T3 are indeed sufficient to promote a decrease in TRH gene expression. The reduction in steady-state concentrations of ppTRH mRNA was dose dependent, whereas the levels of pIns mRNA were unaffected by T3.

The release of pTRH-(160–169) is, like that of TRH and insulin, unaffected by short term T3 treatment. Chronic T3 treatment dose dependently inhibited pTRH-(160–169) and insulin release and selectively affected TRH content. The inhibition of insulin release is not due to a decrease in the insulin store (unchanged) or to a membrane effect (unaffected), as shown by the short term release experiments. The similarity of the secretory patterns of pTRH-(160–169) and insulin suggests that pTRH-(160–169) is involved in insulin secretion. A direct test is now needed to address this question. Taking into account the elevated concentrations of endogenous pTRH-(160–169), one appropriate approach may be immunoneutralization experiments using anti-TRH-(160–169) serum. This has been used to determine the effect of islet TRH on glucagon secretion (14). pTRH-(160–169) has also been detected in pituitary cells and has been reported to potentiate the action of TRH on TSH and PRL secretion (8, 9).

T3 selectively down-regulates the expression of the TRH gene, but has no effect on pIns mRNA or insulin contents. In contrast to its effect on islets, thyroid hormone up-regulates the cell contents of ppTRH mRNA and TRH in anterior pituitary cells in culture (29), suggesting tissue- or cell-specific regulation of the TRH gene.

The T3-dependent inhibition of TRH gene expression in cultured islets is consistent with recent data obtained with hypothyroid rats. In this in vivo experimental model, the steady-state concentrations of islet ppTRH mRNA and TRH contents markedly increased, and T3 replacement restored the euthyroid levels (16). We also found twice as much basal secretion of TRH in isolated perfused pancreas from hypothyroid rats as in euthyroid pancreas, but significantly less insulin secretion (data not shown). Therefore, the regulation of TRH in fetal islets culture by T3 mirrors that of the adult islets from hypothyroid rats, except for the secretory patterns. This difference may be due to the functional immaturity of fetal islets. The regulation of islet TRH release by glucose is also the opposite pattern to that of insulin in the adult islets, but similar to that of insulin in the neonatal islets in situ and fetal islets in culture (10).

Experimental hypothyroidism is associated with increased ppTRH mRNA (30, 31) and TRH release (32, 33) in the rat hypothalamus. These thyroid status-associated effects are all reversible by T3 replacement, indicating that they were mainly due to the circulating thyroid hormone. Consistent with this, unilateral implants of T3 directly into the hypothalamus regulate TRH synthesis by negative feedback (34).

Interestingly, the hypothyroidism produced by targeted disruption of the ppTRH gene also involves a significant decrease in insulin secretion, but thyroid hormone replacement does not correct the deficit in insulin secretion (35).

It is not known whether T3 regulates TRH gene expression transcriptionally, by changing ppTRH mRNA stability, or both. There are, however, several arguments for a classic genomic action of T3: 1) T3 causes parallel decreases in TRH, pTRH-(160–169), and ppTRH mRNA levels, indicating that the effect is probably not posttranslational; 2) T3 has no acute effect on the content or/and release of TRH and pTRH-(160–169) during short term incubations, suggesting that T3 acts primarily at the transcriptional level; and 3) we have previously shown that there are unpaired TRH-degrading activity (TRH-DA) and high concentrations of TRH in the hypothyroid rat pancreas (17, 18). We have also shown that administering T3 in vivo increases TRH-DA and decreases the TRH content (36, 37). Similar decreases in TRH content are observed with purified fetal islets, almost free of degrading enzymes, so the initial impact of T3 is probably at the transcriptional level, although fine regulation of the TRH concentration by TRH-DA cannot be excluded. The decrease in
islet pTRH mRNA mirrors at least partly that of the islet TRH and pTRH-(160–169) contents and releases. 4) The presence of nuclear T3 receptors in the pancreas (38) suggests that T3 has a direct effect on the islet TRH gene. Furthermore, T3 receptor-binding site consensus sequences have been identified in the TRH promoter that may be the target of the direct action of T3 (39–41). T3 may differentially regulate TRH gene expression by inhibiting or stimulating a trans-acting factor (42) or by tissue-specific regulation of separate thyroid hormone receptor subtypes (43). The mechanisms underlying this differential regulation have yet to be investigated. The pathophysiological relevance of the effect of thyroid hormone on islet function and development is unknown.

This study, by documenting the thyroid-dependent regulation of developing islets, provides the basis for examining the nuclear T3 effect on the ontogeny of pancreatic hormones. This concept has been investigated separately using fetal islets transfected with TRH regulatory sequences.

References


