LECTURE

Thyrotropin Releasing Hormone (TRH) may preserve pancreatic islet cell function: potential role in the treatment of diabetes mellitus

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Abstract. Thyrotropin Releasing Hormone (TRH), first identified in the hypothalamus as a regulator of the Pituitary-Thyroid axis, has also been found in the β-cell of the pancreas co-localised with insulin. The significance of this association is emphasised by the report that the TRH knock-out (KO) mouse is hyperglycemic. These findings have led to speculation that TRH may have a physiologic role in the regulation of carbohydrate metabolism. To understand better the role of TRH in the pancreas, TRH was administered to rats rendered diabetic from streptozotocin damage to the islets of Langerhans. This resulted in almost complete normalisation of the profound hyperglycemia. TRH is capable of reversing Diabetes Mellitus (DM) in an experimental animal model, possibly by promoting neogenesis of beta cells through induction of adult stem cells in the pancreas. These studies point to a potential therapeutic role for TRH in the treatment of DM in man. (www.actabiomedica.it)

Key words: Thyrotropin-Releasing Hormone (TRH), pancreas; islets of Langerhans, diabetes mellitus, streptozotocin, insulin, stem cells

Introduction

Thyrotropin-releasing hormone (TRH), (L-pyroglutamyl-L-histidyl-L-prolineamide), located in central nervous sytem (CNS) as well as other regions of the body, acts as a neurotransmitter or neuromodulator (1). TRH is released from its location in the hypothalamic nerve terminals in the median eminence to stimulate the secretion of thyroid stimulating hormone (TSH), the function for which TRH is named (2). Additionally, TRH is also synthesized in the Islets of Langerhans and localized in the insulin-producing β-cells (3). Unlike insulin and glucagon, the highest concentrations of TRH and other pro-TRH derived peptides are expressed during the early development of the neonatal rat pancreas (4) and human fetal pancreas (4), rather than in the adult. It has been reported that pancreatic TRH is involved in stimulation of glucagon release and inhibition of exocrine pancreatic se-

cretion (5). However, central to this review is a) the capacity of the tripeptide to potentiate glucose-induced insulin release and b) the effect of TRH gene knockout (KO) in experimental mice, which results in hyperglycemia (6). Such findings suggest that TRH may play an important role in carbohydrate metabolism, with its disruption leading to the development of Diabetes Mellitus.

Expression of TRH in the pancreas

In the pancreas TRH is expressed in the β-cells of the islets of Langerhans, where it colocalizes with insulin in the same granules (7). The levels of TRH are high during the neonatal period and decrease rapidly as postnatal development progresses (4). Both in the rat and mouse, insulin levels increase between E12 and E14 (a period called the primary transition) (4, 8). The

levels remain quite stable between E14 and E16 but increase very rapidly thereafter, during a period called the secondary transition; at this time TRH is expressed in previous immunochemical stain negative cells counted during the primary transition. Prior to E15, insulin expressing cells contain neither granules resembling those found in mature β-cells nor Rab3A and SNAP-25, two molecules important for the control of insulin secretion. Moreover, prior to E15, there is a negative stain for the glucose transporter Glut2 in insulin-expressing cells. However, after E16, cells staining for insulin now express TRH. Although TRH has been proposed as a marker for insulin maturation, the fact that a large number of molecules including Glut2, Pdx-1 and proTRH are absent from insulin containing cells which develop early during development suggests that these factors may be inducers for precursor cells developing insulin secretion in response to glucose suggesting that TRH is necessary in the prenatal period for full maturation of insulin secreting islet cells.

Effects of TRH on pancreatic insulin secretion

TRH regulates blood glucose

While the best-characterized physiologic role of TRH is regulation of TSH and, thereby thyroid hormone secretion, TRH has several other biologic effects, including regulation of neuronal growth (9), recovery from spinal cord injury (10), appetite control (11) and alcohol consumption (12). Serial studies indicate that TRH has an anti-hyperglycemic action *in vivo* presumably via the CNS (6, 13-16). Since TRH exerts an anti–hyperglycemic effect even in animals with a hypophysectomy, neither the pituitary-thyroid axis not any other hormone released from the pituitary plays a role in this phenomenon (17). Of note, CNS administration of TRH fails to reverse hyperglycemia in streptozotocin-induced diabetic rats indicating that the effect of TRH is ultimately mediated via insulin. Targeted disruption of the TRH gene in mice results in hyperglycemia. However, administration of thyroid hormone for the concomitant hypothyroidism does not reverse the hyperglycemia, which thus results from the absence of TRH itself and not thyroid hormone.
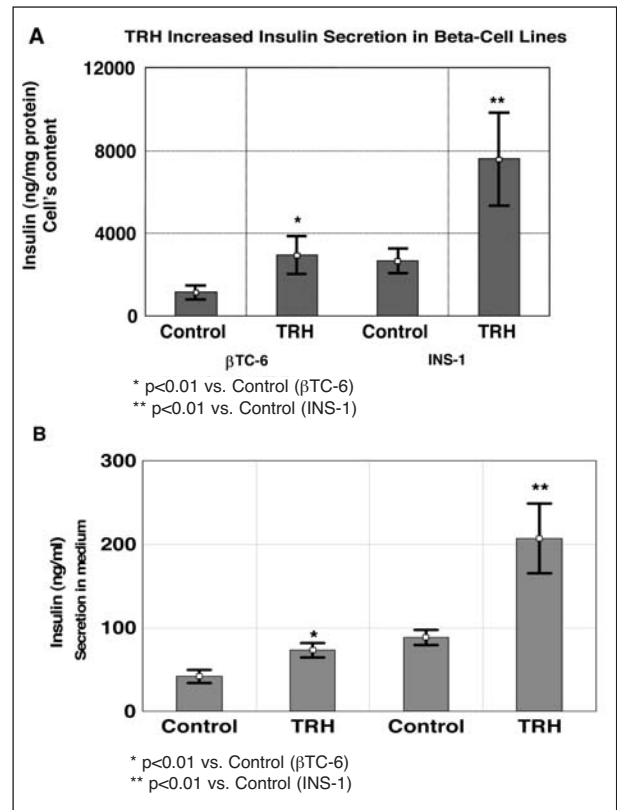
Figure 1. Insulin levels in βTC-6 (a mouse derived pancreatic beta cell line) and INS-1 (rat insulinoma cell line) cell extracts and medium after exposure to TRH: Cells were cultured for 24 hours with or without TRH (n=6 each group). Culture medium was collected and harvested cells were extracted by 5% TCA. Insulin content and secretion were measured by ELISA. Insulin content was normalized relative to protein concentration (mg/ml) in the cell extracts. TRH treated cells contained greater levels of insulin in cell extracts (A), and culture medium (B) vs controls (from reference 25, with permission)

Intravenous injection of TRH in rabbits increases the levels of glucagon and insulin in the blood (18), indicating that TRH can directly stimulate secretion from the endocrine pancreas (Figure 1), while quantitative analysis of pro-TRH mRNA concentrations in fetal islet cultures provides direct evidence of TRH biosynthesis in this location (19).

Pancreatic TRH can stimulate endocrine pancreatic function and/or development

Although it has been reported that TRH may participate in the regulation of pancreatic β-cell deve-

lopment and insulin secretion, the precise biological role of TRH expression in the pancreas remains unclear. Pancreatic TRH production may locally modulate insulin secretion by potentiating glucose-stimulated insulin release as demonstrated in isolated perfusion of fresh islets and islet cell lines (20). TRH, acting as a local modulator of intra-islet hormone secretion, enhances basal glucagon and somatostatin secretion. It has been postulated that there exists a direct effect of pancreatic TRH on glucagon-containing (alpha) cell secretion, which, in turn, may produce fluctuation in (islet cell) somatostatin secretion that can directly inhibit insulin production. In man, TRH administration to a group of patients with hyperparathyroidism led to significantly higher serum levels of insulin and glucagon, which likely resulted from a direct effect on the pancreatic islet. TRH also exhibits a dose-dependent inhibition of carbachol-stimulated amylase secretion, which is eliminated in the experimental model of dissociated acini. These results are consistent with a role for TRH in the paracrine regulation of exocrine as well as endocrine pancreatic secretion. In addition, the reduction of glycodeoxycholic acid-induced pancreatic damage by TRH suggests that TRH has a protective function against toxins in the pancreas.

The discovery of high levels of pancreatic TRH expression during embryonic development suggests that TRH might be involved in pancreatic β-cell development. Dexamethasone treatment results in an increase of pancreatic weight and retardation of the peak pancreatic TRH concentration and islet development, pointing to a role for TRH in β-cell/islet maturation during the phase of pancreatic growth, which is due to hyperplasia (21). Maternal diabetes, induced by streptozotocin injection, reduces TRH levels in the pancreas of newborn rats (22). The associated reduction in insulin secretion is consistent with the importance of TRH for pancreatic β-cell development. The TRH precursor level on the day of birth is 10 fold lower in pups of diabetic rats. The depression of pancreatic TRH was less profound 24 hours later, and by postnatal day 5, elevated TRH levels were observed (22). Thus, postnatal TRH expression in the pancreas of pups is retarded by maternal diabetes and compensatory TRH over-expression may be necessary for pancreatic islet formation (8). These lines of evidence all indicate that TRH

plays a critical role in pancreatic β-cell/islet proliferation and maturation in the neonatal period.

TRH receptors (TRH-R) in mouse pancreatic islets and HIT-T15 (HIT) cells, a hamster clonal β-cell line, were identified by a RT-PCR study (23). The results showed significant expression of TRH-R subtype 1 (TRH-R1) mRNA in both mouse pancreatic islets and HIT cells, but no expression of TRH-R subtype 2 (TRH-R2) mRNA, which is expressed predominantly in the CNS. TRH-R1 in the pancreas shares 93.3 % homology with that in the pituitary. A signal of an approximately 3.7kb band was identified by Northern blot analysis. Functional tests showed affinity of TRH-R1 for TRH in β-cells with various Kd values (23). TRH significantly increased β-cell intracellular calcium concentration, which was not affected by removal of extracellular calcium (24). Recent studies in our laboratory verify TRH-R1 expression in rat-derived β-cell lines as well as whole pancreas that included non-islet tissue (25) (Figure 2). TRH receptor activation was linked to EGF receptor phosphorylation (25). The presence of TRH-R1 in the pancreatic β-cell is consistent with the regulation of pancreatic function by TRH through an autocrine or paracrine mechanism.

Regulation of TRH in the pancreas

Insulin inhibits, while glucose stimulates, endogenous TRH release from pancreatic islet cells *in vitro*, the mechanism for which may involve cellular cAMP production regulated by somatostatin (26, 27). We propose that there is an insulin-TRH-glucose feedback mechanism which is linked with somatostatin to indirectly modulate β-cell regeneration. This may partially explain why in tissue culture pure β-cells do not survive as well as when they are part of an entire islet.

Effect of TRH on pancreatic microenvironment by alteration of gene expression within the pancreas

A recent study by Yano and Luo (28) indicated that TRH influences the rat pancreas through expres-
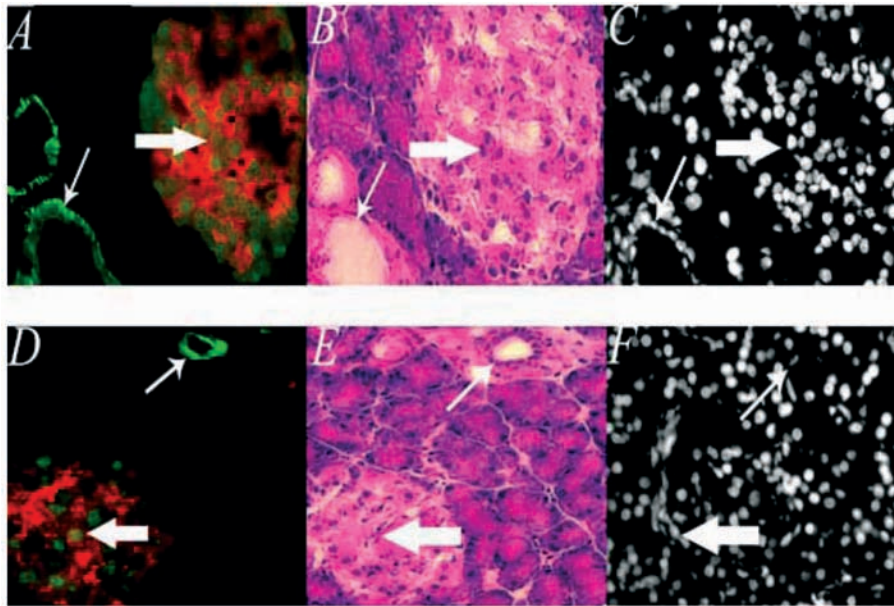
Figure 2. In situ hybridization of TRH-receptor-1(TRH-R1) in rat pancreas: A and D) Dual fluorescent image of rat pancreas. Red indicates insulin immunofluorescence; Green indicates TRH-R1 *in situ* hybridization. B and E) shows hematoxilin and eosin staining for tissue morphology; C and F) shows DAPI for nuclei staining. The large arrows indicate the yellow color, a mixture of green and red represents colocalization of insulin and TRH-R1 in islet and the small white arrows indicate the positive staining of TRH-R1 in epithelial (A) (B) and ductal (D) (E) cells (from reference 25, with permission)

sion of multiple functional genes *in vivo*. TRH upregulated 29 genes in the pancreas and 31 genes in a rat derived pancreatic β-cell line, INS-1 cells, which included G-protein coupling receptor related genes (GPCR kinase 4 and 5, transducin-β1subunit, Arrestin-β1, transducin-β1), Ca^{2+} channel enhancers (Ca^{2+}/calmodulin-dependent protein kinase, type I and II), protein kinases (serine/threonine kinase-3, PKCβ, PCTAIRE-3, v-mos) and proliferation or differentiation signal transduction related genes (MAPK3, growth factor receptor-bound protein 2, n-myc, GAP-43), and down-regulated pro-apoptotic Bax gene. Noticeably, TRH significantly stimulated insulin secretion genes (N-methyl-D-aspartate receptor-2A, GABA-A receptor, RAB2, Ras-related GTPase and ADP ribosylation factor 1 and 5). Of note, there is a difference in gene expression between the pancreas and INS-1 β-cells, which included 6 initiated and 14 turned off genes from signal transduction group plus one initiated anti-apoptotic BcLX gene from the 36 initiated and 36 turned off genes in pancreas while only 4 genes were initiated and 4 genes

turned off from the 34 signal transduction genes in INS-1 β-cells. We postulate that TRH maintains normal insulin secretion to meet the needs of glucose homeostasis as a consequence of its regulation of genes in the β-cell as well as the whole pancreas. Multiple groups of genes regulated by TRH in the adult pancreas may reflect TRH modulation of a microenvironment which creates a condition for balance of pancreatic endocrine and exocrine cell function.

Future directions

It has been shown that islet cell function of the rat recovers from a 90-95% pancreatectomy following administration of glucagon-like peptide 1 (GLP-1) (29), indicating that either regeneration of new β-cells or enhanced function of remaining β-cells can occur in an impaired pancreas under such a stimulus. Additionally, in rats rendered profoundly hyperglycemic following streptozotocin treatment, which severely damages β-cells in the islets, administration of TRH

is capable of lowering the blood glucose to near normal, presumably by reactivating the capacity to synthesize and secrete insulin, either from residual or newly formed β-cells (30). These reports indicate that TRH and GLP-1 can reverse certain models of Diabetes Mellitus in experimental animals.

As mentioned earlier, TRH may be an inducer of precursor cells (which are possibly adult stem cells present in pancreas) to differentiate into functional β-cells. Before this could be applied therapeutically to Diabetes Mellitus in man, several criteria would need to be met. Ideally, pancreatic β-cells should be differentiated from stem cells that multiply in culture, reproduce themselves exactly, and are self-renewing. Stem cells should also be able to differentiate *in vivo* to produce the desired type of cell. For diabetes therapy, it is not clear whether production of only β-cells will be desirable or whether other pancreatic islet cell types are also necessary. Isolated β-cells -those cultured in the absence of other types of islet cells- are less responsive to changes in glucose concentration than are intact islet clusters made up of all islet cell types. Islet cell clusters typically respond to higher-than-normal concentrations of glucose by releasing insulin in two phases: a quick release of high concentrations of insulin and a slower release of lower concentrations of insulin. In this manner the β-cells can fine-tune their response to glucose. Therefore, it will be preferable to develop a system in which stem or precursor cell types can be cultured to produce all the cells of the islet cluster in order to generate a population of cells that will be able to coordinate the release of insulin in physiologic levels appropriate to the ambient concentrations of glucose in the blood. We believe that treatment of diabetes in the future might be met by *in vivo* initiation of stem cell regeneration for recovery of pancreatic endocrine function, utilizing TRH as an initiator of islet neogenesis as a means of recovering β-cell function (30).

Conclusions

On the basis of current information concerning the role of TRH on glucose homeostasis, it is possible that this neural peptide may not only regulate islet β-cell insulin secretion or synthesis but also modulate pancreatic microenvironment to facilitate endocrine and exocrine cell regeneration and play a β-cell preserver role. Similar to the gut peptide GLP 1, which has been reported to regenerate β-cells, TRH may be a potential adult stem cell inducer causing differentiation into functional cells in a damaged pancreas (31). Such findings suggest that TRH may have a unique role to play as a therapeutic agent in Diabetes Mellitus.

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