Chapter 7

Thyrotropin-Releasing Hormone (TRH) a Small Molecule in Pancreas Promotes Insulin Producing Cell Proliferation

John Z.Q . Luo, Souriya Vang, Zhao Ting, Ivor Jackson and LuGuang Luo

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http://dx.doi.org/10.5772/57550

1. Introduction

Neural tripeptide amide L-pyroglutamyl-L-histidyl-L-prolineamide (L-PHP, Thyrotropin-releasing hormone, TRH) is a fine molecular peptide that was first identified in the Central Nervous System (CNS) and discovered in many other regions of body later as a neuropeptide hormone or neuromodulator [1]. L-PHP stimulates the thyroid stimulating hormone (TSH) after it is released from the hypothalamic nerve in the median eminence. L-PHP was named as its functional action-TRH [2]. Beyond neuronal tissue, expression of L-PHP was also found in the pancreatic islets where it identifies to the Langerhans-insulin-producing beta cells [3]. However, L-PHP expression and production is significantly different from its production in the nervous system; it is primarily expressed during the early developmental period in rat [4] and human fetal pancreatic tissue [4]. L-PHP stimulates glucagon release and inhibits other pancreatic secretion other than TSH [5]. In this review, based on evidence found in L-PHP gene knockout animal models and its function in regulating insulin release in pancreatic tissue [6], L-PHP may play an important role in carbohydrate metabolism and pancreatic L-PHP disruption may lead to the development of diabetes mellitus.

Expression of L-PHP in the pancreas: L-PHP is expressed in the insulin granules of β cells in pancreatic islets, [7] with high levels during the neonatal period but significantly decreased as postnatal development progresses [4]. A Comparison with L-PHP expression, in the primary transition period between E12 and E14, shows insulin secretion in both rat and mouse while L-PHP remains unexpressed[4, 8]. During this period, insulin stained cells do not express any Rab3A, SNAP-25 (two molecules important for the control of insulin secretion) nor Glut 2 and

granules resemble β cells. However, at E16, L-PHP expression was found and thereafter, high expression of molecules such as Glut2 and Pdx-1, which are necessary for insulin production, maturation and full insulin cell function, were found in the insulin and L-PHP positive cells. L-PHP's significant expression coincides with factors for insulin production, maturation and insulin cell development suggesting that L-PHP is critical for insulin cells as they become functionally mature during early development.

2. Effects of L-PHP on pancreatic insulin secretion

Beyond regulating TSH, L-PHP is also found to be involved in the regulation of neuronal growth [9], facilitating spinal cord injury recovery [10], appetite control [11], and alcohol consumption [12]. The most important role of L-PHP is considered to be its regulation of blood glucose levels *in vivo*, presumably *via* the CNS [6, 13-16]. L-PHP's anti-hyperglycemia function was identified by eliminating pituitary-thyroid axis by a hypophysectomy, which also eliminated other hormones released from pituitary, and suggests its anti-hyperglycemia function beyond its activation in CNS [17]. In another experiment, pancreatic beta cells were destroyed by Streptozotocin and CNS administration of L-PHP failed to reverse high blood glucose, supporting this notion of function outside of CNS activation. L-PHP regulating blood glucose may have a direct effect in pancreatic beta cell instead of *via* CNS or thyroid hormone, which was supported by application of thyroid hormone in hypothyroidism of hyperglycemic animal but did not reverse high blood glucose. Blood glucagon and insulin level was increased by intravenous injection of L-PHP in rabbit [18] and cultured fetal islet identified L-PHP expression by a quantitative analysis [19] which supports the possibility of L-PHP's direct effect on pancreatic beta cell function.

Pancreatic L-PHP can stimulate pancreatic endocrine function and/or endocrine cell development. The mechanisms as to how L-PHP regulates pancreatic β-cell development have not been identified. Gathering evidence from *in vivo* and *in vitro*, we propose that L-PHP may modulate insulin secretion directly when glucose stimulates β-cell, which was demonstrated in isolated perfusion of fresh islets [20] and islet cell lines (Fig. 1). The mechanisms may relate to L-PHP regulating glucagon-containing (alpha) cell secretion resulting in eliminating somatostatin (r-cells) and inhibiting insulin production. A clinical study in hyperparathyroidism patients showed that L-PHP application to these patients significantly elevated serum levels of insulin and glucagon and it also had a dose-dependent inhibition of carbachol-stimulated amylase secretion, suggesting a role for L-PHP in the paracrine regulation of exocrine as well as endocrine pancreatic secretion.

L-PHP protects pancreatic tissue from damage and toxins like the reduction of glycodeoxycholic acid. Evidence suggests that L-PHP plays a critical role in β-cell maturation. During the phase of pancreatic development, which includes high levels of L-PHP expression in early pancreatic β-cell development, dexamethasone treatment eliminated the L-PHP peak and resulted in retarded β-cell development [21]. Also, newborn rats were found to have reduced L-PHP levels due to maternal diabetes caused by streptozotocin (STZ) injection [22]. The

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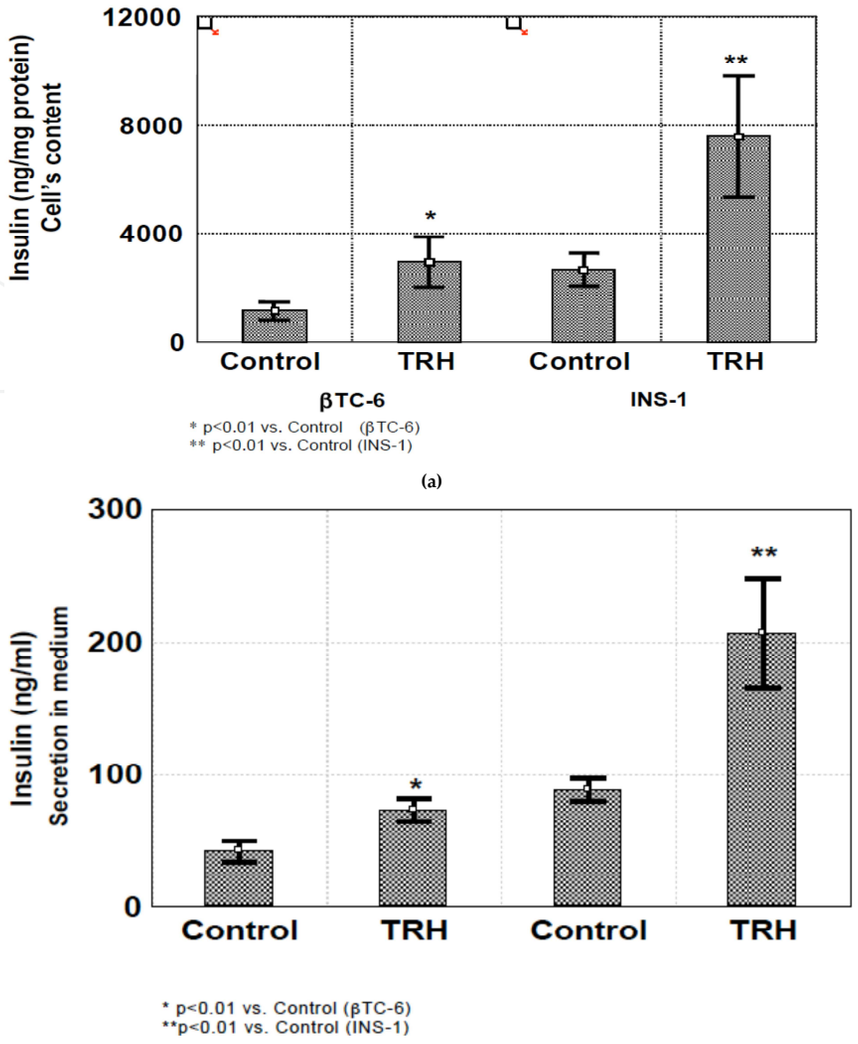

Figure 1. Insulin levels in βTC-6 (a mouse derived pancreatic β cell line) cell extracts and medium after exposure to TRH Cells were cultured for 24 hours with or without TRH (n=6 each group). Culture Medium was collected and harvested cells were extracted by 5% TCA. Insulin content and secretion were measured by ELISA. Insulin content was normalized relative to protein concentration (mg/ml) in the cell extracts. L-PHP treated cells contained greater levels of insulin in cell extracts A and culture medium B vs controls. (From reference #25, with permission)

observation of ten-fold lower L-PHP in pups of diabetic rat followed by a postnatal day 5 elevation of L-PHP reducing blood glucose levels [22] suggest that L-PHP expression during β-cell development is important and it may prevent diabetes from developing in later life.

The L-PHP receptor consists of two major sub-types (R1 and R2, recently identified third type). Using RT-PCR, receptor R1 is identified as expressed in HIT-T15 (HIT) cells, a hamster clonal ß-cell line [23], and mouse pancreatic islets, but expression of R2 is not found. R2 was identified as expressed predominantly in the CNS, but not other tissue. By northern blot analysis it was found that R1 in pancreas is of 3.7-kb size and shares 93.3% homology with that in the pituitary. Evaluation of R1 function by receptor affinity found various kDa values in ß –cells [23]. ß –cell intracellular calcium concentration was significantly increased by L-PHP and removal of extracellular calcium does not change this effect [24]. Our group work has shown

R1 expression in rat-derived β-cell lines as well as whole pancreas that included nonislet tissue [25]. R1 receptor was also found to associate with EGF receptor function called cross linking [25] (Fig. 2).

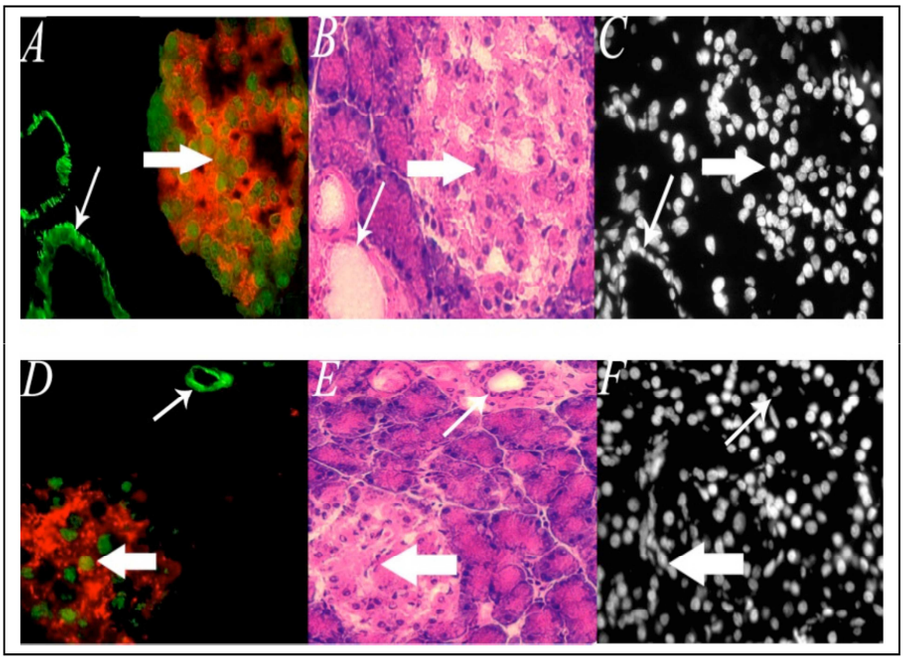

Figure 2. In situ hybridization of L-PHP-receptor-1(R1) in rat pancreas A. and D. Dual fluorescent image of rat pancreas. Red indicates insulin immuno-fluorescence; Green indicates R1 in situ hybridization. B. and E show H&E staining for tissue morphology, C and F show dapi for nuclei staining. The large arrows indicate the yellow color, a mixture of green and red represents colocalization of insulin and R1 in islet and the small white arrows indicate the positive staining of R1 in epithelial A. B. and ductal D. E. (From reference #25, with permission)

3. Regulation of L-PHP in the pancreas

In vitro studies have shown that L-PHP is stimulated by glucose and suppressed by insulin release. Cellular cAMP production regulated by somatostatin may involve glucose and insulin regulation of L-PHP [26, 27]. We hypothesize that there is an α-β-γ integrating system, which releases insulin-L-PHP-somatostatin coordinated in respond to glucose challenge in islet. To support this hypothesis, it needs further study but evidence in that tissue cultures of pure β cell do not function as well as an entire islet may be part of the support.

4. L-PHP alteration of gene expression modifys microenvironment within the pancreas

Pancreatic microenvironment alteration by L-PHP has been reported [28]. The findings show that multiple functional genes in rat pancreas were influenced by L-PHP *in vivo*. A total of 60

genes are found to be regulated by L-PHP, 29 genes in the pancreas and 31 genes in rat derived pancreatic β-cell line, INS-1 cells. These genes include Ca2+channel enhancers (Ca2+/calmodulin-dependent protein kinase, type I and II), G-protein coupling receptor related genes (GPCR kinase 4 and 5, transducin-β 1 subunit,Arrestin-β1, transducin-β1), Protein kinases (serine/threonine kinase-3,PKC β, PCTAIRE-3, v-mos), 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. Genes relative to insulin secretion are significantly increased by L-PHP including N-methyl-D-aspartate receptor-2A, GABA-A receptor, RAB2, Ras-related GTPase and ADP ribosylation factor 1 and 5. The differential gene expression between β-cells and total pancreatic tissue in response to L-PHP shows that of the 36 genes that are initiated and 36 genes that are turned off relative to signal transduction. In rat pancreas 6 genes were initiated and 14 genes were turned off, with one initiating the anti-apoptotic BcLX gene. While in rat INS-1 β cell line only 4 genes were initiated and 4 genes turned off from the 34 signal transduction genes. These significant variations between pure β-cell and entire pancreatic tissue indicate that L-PHP can regulate β-cell function by directly working on β-cells or by indirectly altering pancreatic microenvironment to maintain and facilitate β-cell response to glucose resulting in a balance *in vivo* of glucose metabolism.

5. Regulation of β-cell proliferation by signal pathways from L-PHP to growth hormone activity in pancreatic islet

L-PHP has been reported to stimulate R1 and dissociate the GPCR complex, activating protein kinase C [29] and mitogen-activated protein kinase (MAPK) [29] in both a PKC-dependent and a PKC-independent manner in the neuronal cell lines [30]. These effects may involve activation of tyrosine kinase, which leads to the activation of Ras and MAPK cascade. The signaling pathways initiating from G-coupled L-PHP receptor in activating MAPK may overlap with the receptor tyrosine kinases activating the Ras-MAPK cascade [31, 32]. There is evidence that L-PHP and EGF have overlapping activities [33] leading to the stimulation of tyrosine phosphorylation of EGF receptors in GH3 cells, a pituitary cell line [34]. L-PHP-induced EGF receptor phosphorylation led to the recruitment of adapter protein Grb2 and Shc in GH3 cells. The hypothesis that L-PHP would activate EGF receptors in β cells through multiple pathways is tested, and data indicated that L-PHP trans-activates EGF receptors through several intra- and extracellular pathways, which are distinguished from pituitary-derived cell lines. R1 can initiate multiple signal transduction pathways to activate the epidermal growth factor (EGF) receptor in pancreatic β cells [35]. By initiating R1 G-protein-coupled receptor (GPCR) and dissociated $\alpha\beta\gamma$ complex, L-PHP (200nM) activates tyrosine residues at Tyr845, (a known target for Src) and Tyr1068 in the EGF receptor complex in an immortalized mouse β-cell line, βTC-6. Through manipulating the activation of Src, PKC and heparin-binding EGF-like growth factor (HB-EGF) with corresponding individual inhibitors and activators, multiple signal transduction pathways linking L-PHP to EGF receptors in βTC-6 cell lines have been revealed. The pathways include the activation of Src kinase and the release of heparin-binding EGF as a consequence of MMP3 activation. Alternatively, L-PHP inhibited PKC activity by reducing

EGF receptor serine/threonine phosphorylation, thereby enhancing tyrosine phosphorylation. L-PHP receptor activation of Src may have a central role in mediating the effects of L-PHP on the EGF receptor (Fig. 3). The activation of the EGF receptor by L-PHP in multiple circumstances may have important implications for pancreatic β cell biology. Since EGF receptor expression has been found to have a high activity during the embryonic developmental period [4, 36], the possibility exists that L-PHP activation of EGF receptors in pancreatic β cells may play a role in β-cell development.

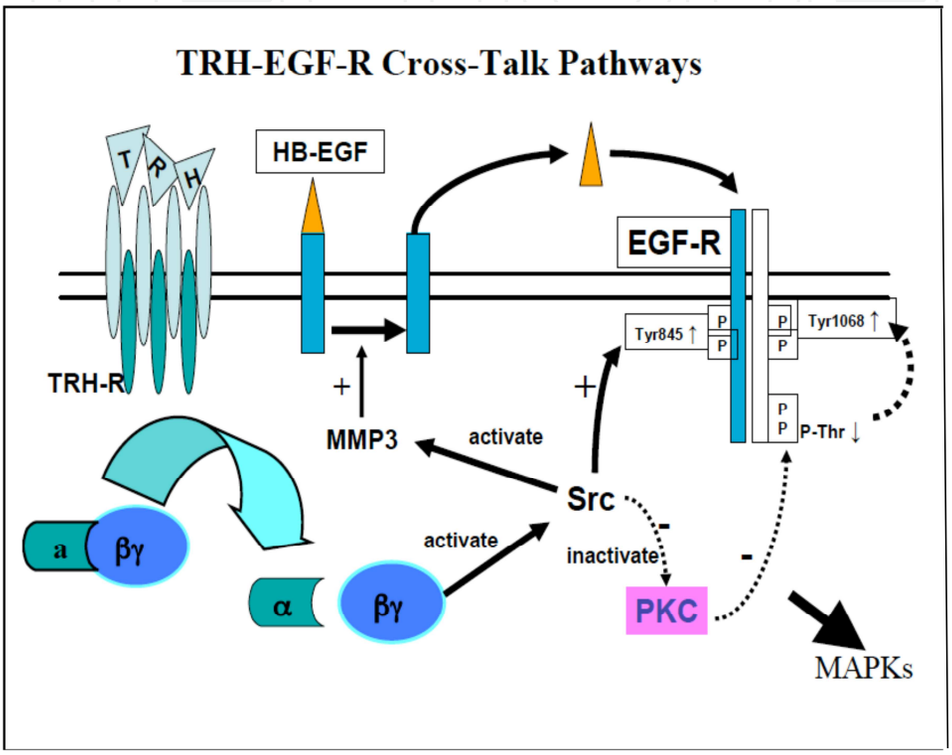

Figure 3. The scheme summarized the mechanism of L-PHP cross talk with EGF receptor in pancreatic β cells. L-PHP binds to its receptor and dissociates GPCR αβγ complex into α and βγ units. The βγ unit activation of the Src kinase directly results in phosphorylation of EGF receptor Tyr 845. In addition, Src indirectly stimulates Tyr 845 phosphorylation by activation of MMP3 to release heparin-binding EGF. Meanwhile, activation of Src kinase inhibition of PKC results in reducing serine/threonine phosphorylation which blocks off the inhibition of serine/threonine phosphorylation on tyrosine phosphorylation and indirectly activates Tyr 1068 phosphorylation in EGF receptor. L-PHP activation of EGF receptor phosphorylation results in the activation of cellular signal pathways such as MAPKs. The activation of Src may have a central role in mediating the effects of L-PHP on the EGF receptor. (R=receptor; _____=activation;------=suppression) (From reference #35, with permission)

6. Conclusions

The small sized L-PHP neuropeptide may play a significant role in direct regulation of pancreatic β-cell function and, through modulation of pancreatic microenvironment, support β-cell survival. The role of L-PHP may be similar to that of the gut peptide GLP-1, that increases β-cell regeneration, but may also have a role in inducing adult stem cell differentiation into

functional β-cells during pancreatic tissue injury, which may be significant for diabetic therapy.

7. Future directions

Rat islet cell function can be recovered 90-95% from a pancreatectomy after application of glucagon-like peptide 1(GLP-1) [38]. This β-cell regeneration from damaged rat pancreas has also been mimicked by STZ damaged rat pancreas following administration of L-PHP [39]. However, human islet β-cell regeneration may differ from rat and it may require a totally different microenvironment. In order to initiate human islet β-cell functional recovery from damage or loss, pancreatic stem cells or stem cells from other tissue, such as bone marrow, must be able to in vivo differentiate into multiple types of endocrine cells (αβγ) to reconstitute a new endocrine system in response to glucose challenge. Initiating L-PHP generation *in vivo* or administration from *in vitro* may be a way to approach this goal. Before the application of this peptide, a series of studies must be performed 1) to prove L-PHP can induce stem cells in the pancreatic environment to differentiate into β-cells and 2) L-PHP can induce other islet endocrine cells, such as α and γ cells, to support and regulate β-cell function, even β-cell regeneration. The current evidence from in vivo animal models and in vitro is very promising and encouraging; still multiple steps are needed before L-PHP can be applied in human diabetic therapy.

Acknowledgements

This work was partially supported by Roger Williams Hospital Research fund, and NIH Grant Number 1R01DK097380-01A1 from National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and National Institute of General Medical Sciences (NIGMS). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

Author details

John Z.Q . Luo[1], Souriya Vang[2], Zhao Ting[2], Ivor Jackson[1] and LuGuang Luo[2*]

*Address all correspondence to: lluo@rwmc.org

1 Brown Alpert Medical School, Rhode Island, USA

2 Department Research, Center of Stem Cell Research, Roger Williams Hospital Providence, Rhode Island, USA

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