Thyrotropin releasing hormone and its cyclised C-terminal peptide inhibit thyroglobulin release from thyroid follicular cells: evidence for acute regulation of hormone production at the thyroid gland

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Abstract

Purpose: Thyrotropin releasing hormone (TRH), a tripeptide hormone produced in the hypothalamus, controls thyroid stimulating hormone (TSH) production from the pituitary gland and hence the production of thyroid hormone. Extra-hypothalamic production and action of TRH has been detected, as has the presence of a C-terminal cyclised derivative, histidyl-proline diketopiperazine (His-Pro DKP). This study investigates the effects of these compounds on thyroglobulin release from thyroid follicular cells.

Methods: DKPs were identified by chromatography and mass spectrometry. Expression of RNAs and proteins were identified in the FTRL-5 thyroid cell line and supernatant using RT-qPCR and immunoblotting.

Results: We show that TRH is expressed by rat follicular thyroid cells, as is Pgpep1, the enzyme required for removal of the N-terminal amino acid of TRH. The rate of His-Pro DKP production from the C-terminal dipeptide of TRH is enhanced by thyroid extract *in vitro*. Both TRH and His-Pro DKP reduce thyroglobulin release from thyroid follicular cells with the magnitude of this effect attenuated in the presence of TSH, which also inhibits the expression of Pgpep1.

Conclusion: Collectively, these data indicate that TRH and its cyclised dipeptide derivative directly regulate thyroid production within the thyroid gland, potentially in a manner dependent upon the activity of the hypothalamic-pituitary-thyroid (HPT) axis. These findings provide further evidence that C-terminal peptide derivatives of classical hormones possess intrinsic biological activities.

Introduction

Thyrotrophin releasing hormone (TRH) is produced in the hypothalamus and acts upon thyrotroph cells in the anterior pituitary to stimulate thyroid stimulating hormone (TSH) production. The TSH travels in the circulation and binds to receptors on thyroid follicular cells to stimulate the release of the T3 and T4 forms of thyroid hormone. Thyroid hormone regulates its own production in a classical endocrine negative feedback loop known as the hypothalamic-pituitary-thyroid (HPT) axis. TRH is a tripeptide hormone with the sequence pyroglutamyl-histidyl-proline-$CONH_2$ (pGlu-His-Pro amide) which is cleaved from the precursor peptide proTRH. TRH has a short half-life of 4–5 minutes [1] and can be metabolised to form His-Pro amide which cyclises to form His-Pro diketopiperazine (His-Pro DKP, also referred to as cyclo (His-Pro)). His-Pro DKP is more stable than TRH and can be found in concentrations significantly higher than TRH [2]. Rather than simply being an inactivation process to regulate TRH levels, His-Pro DKP has been shown to be bioactive. It can inhibit both the release of prolactin from pituitary cells in culture [3] and 2-deoxy-D-glucose-stimulated secretions from the exocrine pancreas [4]. Extrahypothalamic expression of TRH has been documented, with TRH found to be expressed in the human thyroid gland [5], in particular in the parafollicular cells [6], and TRH receptor expression was noted in both the follicular and parafollicular cells [7]. Therefore, we set out to examine the processing of TRH into its cyclic C-

terminal dipeptide, His-Pro DKP, and the ability of these peptides to act on follicular cells to influence the rate of release of thyroid hormone, using the rat follicular cell line FRTL-5.

Our results indicate that FRTL-5 cells express TRH, the TRH receptor, and the enzyme required for the formation of His-Pro amide, pyroglutamylpeptidase 1 (Pgpep1), from TRH. We confirm that His-Pro amide is readily converted to His-Pro DKP with kinetics enhanced by thyroid homogenate, and that both TRH and His-Pro DKP can significantly reduce the secretion of thyroglobulin by FRTL-5 cells. Furthermore, Pgpep1 expression can be inhibited by TSH. Taken together, the results of this investigation point to a new concept for the regulation of the HPT axis by TRH. The tripeptide sequence of TRH is required for the activation of the thyroid hormone axis while the C-terminal dipeptide participates in the regulation of hormone production at the thyroid gland, counterbalancing the overall stimulating activity of the tripeptide to fine tune the production of thyroid hormone, potentially depending on the status of activation of the HPT axis. The evidence thus indicates that a dual mechanism exists for thyroid hormone regulation by TRH and further supports the existence of more wide ranging activities of C-terminal dipeptides derived from classical peptide hormones.

Materials And Methods

His-Pro amide and His-Pro DKP were obtained from Bachem Ltd, Hamburg, Germany. The identity and homogeneity of the synthetic peptides were confirmed by mass spectrometry and high pressure liquid chromatography (HPLC).

An expression plasmid containing a human glutaminyl cyclase cDNA was a gift from CERM [8] and was distributed through the da Vinci European BioBank [9]. Expressed protein was purified by the Queen Mary University of London protein purification facility. The methods used have been described previously [8].

Time course cyclisation of dipeptide amides

Synthetic peptides (10 mg) were incubated at 37ºC in 1 ml of PBS containing 10 mM phosphate at pH 7.2. Aliquots of the reaction mixtures (100 µl) were removed at intervals and the DKP generated was resolved from the dipeptide amide by HPLC. To improve the chromatography of histidine-containing peptides, the pH of each sample was raised above neutrality before injection into the column (C18 Microbondapak, 15 x 0.5 cm). The elution was performed at a flow rate of 1ml/ min using a mobile phase of 0.1% trifluoracetic acid (TFA)-doped water and acetonitrile doped with 0.1% TFA in a linear gradient over 30 min. The samples were frozen at -80ºC to prevent adventitious cyclisation taking place during storage. The identity of DKP produced was confirmed by co-chromatography with the corresponding synthetic peptide and by mass spectrometry.

Enzyme-catalysed cyclisation was performed at 37^{o}C in the presence of a preparation of glutamine cyclase (50 µl of a solution containing 2 mg protein/ ml of 10 mM phosphate) and incubation was carried out under the conditions described for the enzyme-free reaction.

Time course incubation of dipeptide amides was also performed together with an extract of pig thyroid. To prepare the extract, the tissue (10 mg) was homogenised in 7 ml of PBS and the suspension centrifuged at 5000 rpm for 5 min. The supernatant was decanted and incubation of peptides (2 ml of a solution containing 10 mg of peptide in PBS) was carried out together with 2 ml of the enzyme supernatant. Aliquots (400 µl) were taken at different time intervals and cyclisation terminated by freezing. The amounts of DKP and dipeptide amide were assigned from their optical densities at 220 nm after resolution of the peptides by HPLC.

FRTL-5 cell culture

The thyroid rat cell line FRTL-5 (ATCC CRL 8305) was cultured in Nutrient Mixture F-12 Coon's modification (F6636) supplemented with sodium bicarbonate (2.68 g/l, S5761), insulin (10 µg/ml, 91077C), hydrocortisone (10 nM, H0888), transferrin (5 µg/ ml, T8158), Gly-His-Lys acetate (10 ng/ ml, G1887), somatostatin (10 ng/ ml, S9129), 5% Foetal Bovine Serum and optionally TSH (10 mU/ ml, 609385, Calbiochem). This formulation was named 6H media with, and 5H media without, TSH [10]

RT-PCR and real time quantitative PCR

RNA was harvested from FRTL-5 cells using an RNeasy mini kit (Qiagen) and converted to cDNA using a QuantiTect Reverse Transcription Kit (Qiagen). Amplicons were purified using a QIAquick gel extraction kit (Qiagen) and sequenced by Eurofins. QPCR was performed with SYBR green using a KAPA SYBR kit and samples were run on a Stratagene MX3000 cycler and analysed using MXPro software. GAPDH was used as a control. Primers used were: GAPDH forward (F) TGCACCACCAACTGCTTAG, reverse (R) GGATGCAGGGATGATGTTC; rat proTRH F ATTCTCGTGGAAAGACCTCCAGC, R GACATCTGAGAACCAGGAATCCA; rat TRH-R1 F AGATGTTTCAACAGCACCGTTTC, R TCTGTGCTAAAGCGGTCTGACTC; rat TRH-R2 F GGTTCTTCCTGGTGGATCTCAAT, R GAGCAGTACCAGTGTGCGGTAAG; rat Pgpep1 F TAGAGAAACTGGGCCTTGGG, R ACCGGCAATTATCCAGTCCT.

His-Pro DKP and TRH modulation of thyroglobulin release

FRTL-5 cells were cultured in 5H or 6H media for 72 hr in 24 well plates at a density of 10^5 cells per well in 1 ml media prior to the secretion assay. Cells were washed three times with PBS (1 ml) to remove serum traces and were treated with1 µM His-Pro DKP (4003655.0050, Bachem) or 3 µM TRH (P1319, Sigma Aldrich) in 5H, 6H or serum free media (all supplemented with 0.01% bovine serum albumin (BSA) to act as a western blotting loading control) from 5 min to 24 hr. The reactions were terminated by placing the tissue culture plates on ice. Media (500 µl) were collected from each well, centrifuged at 1500 g to remove detached cells and cell debris and 10 µl aliquots were mixed with 6X Laemmli buffer in a SDS-PAGE gel for immunoblot analysis.

Protein electrophoresis and immunoblot

10 µl of supernatants mixed with 2 µl 6X SDS-PAGE were boiled for 5 min and loaded in a NuPAGE 4–12% Bis-Tris Gel 1mm, 15 well (NPO323, Thermofisher). Electrophoresis was set at 20 volts for 20 hr

followed by 100 volts for 2 hr. Wet transfer from gel to Nitrocellulose blotting membrane (10600002, GE Health Care) was set at 20 volts for 20 hr. Membranes were blocked with 5% semi-skim milk in PBS-Triton X100 (0.05%) for 1 hr and incubated with primary antibodies 1:10^4 thyroglobulin (156008, Abcam) produced in rabbit and 1:10000 Monoclonal Anti-BSA antibody produced in mouse (B2901, Sigma Aldrich). The latter was used as a loading control. After 3 washes of 5 min with PBS-Triton X100, secondary antibodies (Goat anti-rabbit (925-32211 800CW, Licor) and Goat anti-mouse (L 925-68070 680RD, Licor)), at a 1:5000 dilution, were incubated with the membrane for 30 min, membranes were washed three times in PBS-Triton X100, twice in PBS and then scanned in a near infrared scanner (Odyssey, Licor).

Statistical analysis

Three biological replicates or more for each condition were included in each experiment which was replicated twice. A Kruskal-Wallis test was used for statistics and p-values below 0.05 were considered statistically significant.

Results

FRTL-5 cells express TRH, TRH receptors and Pgpep1

To characterise the FRTL-5 cell line, which was our model for a thyroid follicular cell, RNA was harvested and subjected to RT-PCR with primers specific for TRH and the rat TRH receptor isoforms 1 and 2. We were able to detect products with primers against proTRH and TRH-R2, but not TRH-R1 (Fig. 1a), and these amplicons were sequenced and gave 100% identity with their expected gene product (data not shown). These data suggest that rat follicular cells can both produce and respond to TRH. In order to form His-Pro amide from TRH, the N-terminal pyroglutamyl amino acid has to be cleaved from the tripeptide and this is catalysed by the enzyme Pgpep1 [11]. RT-PCR detected a specific product for this gene in FRTL-5 cells (Fig. 1b) and the amplicon was confirmed as having 100% identity (data not shown).

Cyclisation of His-Pro amide

FRTL-5 cells, by expressing TRH and Pgpep1, have the ability to produce His-Pro amide. We next investigated the conversion of His-Pro amide into His-Pro DKP by incubating His-Pro amide in 10mM phosphate pH 7.2 over a 12 hr time course. HPLC analysis of the conversion of His-Pro amide to form His-Pro DKP showed that cyclisation took place reproducibly with kinetics corresponding to a half-life of 2 hr (Fig. 2a). At each stage in the reaction, the decline in dipeptide amide could be accounted for by the increase in DKP formed. During incubation for 24 hr at neutral pH, there was no evidence for hydrolysis of His-Pro amide to form His-Pro COOH. These data indicate that cyclisation can occur spontaneously. An enzyme that can specifically catalyse the formation of His-Pro DKP has not been identified but the mechanism catalysed by such a putative dipeptide cyclase might be expected to be shared with glutaminyl cyclase [12] since it would catalyse the condensation of adjacent NH_2 and $CONH_2$ groups. The addition of glutaminyl cyclase to the reaction significantly increased the rate of cyclisation of His-Pro

amide (Fig. 2b). The rate of cyclisation of the enzyme-catalysed reaction at 37^{o}C was significantly greater, with a half-life of less than 10 min. Time course incubations of His-Pro amide were also carried out in the presence of a homogenate of porcine thyroid gland. Aliquots were removed at intervals and the DKP generated was estimated after resolution of the peptides by HPLC. Formation of His-Pro DKP took place more rapidly in the presence of the thyroid extract than in its absence (Fig. 2c).

Effect of TRH and His-Pro DKP on thyroglobulin release in FRTL-5 cells

Monolayer FRTL-5 cells do not produce thyroid hormone [13], so to investigate whether TRH or His-Pro DKP could affect follicular cell function, secretion of the precursor molecule thyroglobulin into the media was assayed in the presence (6H media) or absence (5H media) of TSH. In the absence of TSH, TRH significantly inhibited the release of thyroglobulin, eliciting a greater than 50% reduction in thyroglobulin release at a concentration of 3μM after 15 minutes incubation (Fig. 3a). Given the short half-life of TRH, it was possible that a metabolite of TRH was responsible for this effect. To test this theory, the cells were incubated with His-Pro DKP. It was observed that when the cells were treated in the absence of TSH, His-Pro DKP inhibited thyroglobulin release to a similar degree to the inhibition observed with TRH. When the cells were grown in the presence of TSH, a significant decrease in thyroglobulin accumulation was again observed, but to a lesser degree than seen in its absence (Fig. 3b). The inhibition of thyroglobulin trelease by His-Pro DKP was observed at concentrations as low as 0.01mM (supplemental Fig. 1).

The ability of TRH and His-Pro DKP to inhibit thyroglobulin secretion, and by inference thyroid hormone production, might indicate the existence of a local mechanism to acutely regulate the thyroid hormone axis. The data from Fig. 3 suggest that TSH concentrations could affect this mechanism, perhaps in part by regulating the production of His-Pro DKP. As mentioned above, the identity of a dipeptide cyclase that can produce His-Pro DKP from His-Pro amide is not known; however, it is known that Pgpep1 converts TRH to His-Pro amide. Expression levels of Pgpep1 in FRTL-5 cells were analysed by real time PCR and indicated a significant decrease in the presence of TSH down to 20% of control levels (Fig. 4).

Discussion

TRH has a well-recognised role in the pituitary to stimulate the production of TSH [14], but it has also been detected outside the hypothalamus and pituitary, with proposed roles in tissues such as the pancreas, brain, testis, prostate, ovary and duodenum [15, 16]. Here we show that TRH and its receptor, the TRH-R2 isoform, are expressed in rat follicular cells, strengthening the idea that the thyroid is a source of TRH [5, 6]. The observation that FRTL-5 cells express Pgpep1 suggests that follicular cells are the source of the TRH-like dipeptides found in the thyroid. His-Pro amide spontaneously cyclises to DKP and this is accelerated in the presence of thyroid homogenate. Whilst this does not prove the existence of a specific enzyme in the homogenate, and could be due to the ionic conditions present in the extract, it is reasonable to predict that a glutaminyl cyclase-like enzyme is expressed there and it would be of interest to isolate it.

The results from TRH and His-Pro DKP treatment of FRTL-5 are consistent with a view that the 'secondary' biological activities exhibited by hormone amides result from their C-terminal dipeptides. Indeed, His-Pro DKP has been shown to act as an inhibitor of prolactin release from pituitary cells *in vitro* [3] while its parent peptide TRH fulfils a key role in the regulation of thyroid hormones [14]. The action of TRH within the HPT axis, however, may not be limited to the well-known pathway in which TRH regulates the release of TSH from the pituitary. We observed that TRH inhibited thyroglobulin release in cultured thyroid cells, which is in harmony with previous reports that TRH reduced the levels of cyclic AMP in thyroid slices [17, 18]. Taken together, the evidence supports a view that the TRH that is endogenous in the thyroid, or its DKP, may participate in the control of thyroid activity. His-Pro DKP was found to be as potent as TRH in inhibiting the release of thyroglobulin. Thus, the ability to inhibit thyroglobulin release appears to be an intrinsic property not only of TRH but also its C-terminal dipeptide.

This observation is not unique to TRH; many peptide hormones and all hormone-releasing peptides terminate their peptide chain in an alpha-$CONH_2$ group which is essential for their biological activity. Fragments from the C-terminus of these peptides retain the $CONH_2$ group and it is notable that some have been reported to exhibit an activity distinct from the activity of the parent peptide. For example, the C-terminal fragments of oxytocin possess neuroactivity in the brain distinct from the uterotonic activity of the parent hormone [19] and C-terminal fragments of alpha-melanotropin (alpha-MSH) inhibit the release of alpha-MSH and modify the immune response [20–22]. Similarly, it has been shown that the C-terminal dipeptide of beta-endorphin can inhibit the firing of neurones in the brain stem [23]. Evidence is thus accumulating to indicate that the activity induced by peptide hormones is maintained within physiological limits by opposing mechanisms involving synchronisation of 'activation' and 'inhibition'. It is notable that this dual mechanism concept is in line with the well known bidirectional transmission of signals in the CNS where onward transmission takes place at post synaptic receptors but is balanced by inhibition at the presynaptic receptor, thereby consistent with a common evolutionary origin for the central and peripheral processes.

In cell culture, it was apparent that an inverse relationship existed between the degree of inhibition of thyroglobulin release and the concentration of TSH in the cell supernatant, that is, maximum inhibition of thyroglobulin release was observed in the absence of TSH. Furthermore, Pgpep1 expression was inhibited by TSH, and consequently it could be hypothesised that the inhibitory activity of TRH and its C-terminal dipeptide would be effective during the hyperthyroid state when TSH levels are at a minimum. On the other hand, in the presence of TSH the inhibitory activity of His-Pro DKP was diminished and, it is predicted, so too would be its production, and consequently the inhibition it produces may not have an adverse effect on the hypothyroid state. Overall, the antagonist activity of TRH and its dipeptide in the thyroid appears to be synchronised with the agonist activity of TSH and may serve to limit thyroid production in an acute manner with faster kinetics than are achieved by the long range negative feedback of the thyroid axis achieved by elevated thyroid levels inhibiting TRH production at the hypothalamus. In support of this is the observation that the concentrations of TRH-related peptides present in the thyroid

are much greater in hyperthyroid, when TSH expression would be suppressed, compared with hypothyroid tissues [24].

The mode of action of TRH and His-Pro DKP in the thyroid are unknown, with TRH acting potentially in an autocrine or paracrine manner, given its expression by follicular as well as parafollicular cells. The expression of its receptor in follicular cells indicates the potential for direct action, but there is no known receptor for His-Pro DKP, despite the identification of binding sites in the liver [25], and it could be acting in an intracrine manner (Fig. 5). A number of tripeptides structurally related to TRH occur in endocrine tissues and in some cases the 'TRH-like' peptides are accompanied by TRH [5, 26–29]. Given that TRH is known to lose its N-terminal pyroglutamyl residue in the presence of Pgpep1, it was anticipated that peptides with structures related to TRH would also be susceptible to loss of their N-terminal residue and give rise to the corresponding dipeptide amide. pGlu-Glu-Pro amide and pGlu-Phe-Pro amide have been detected in the prostate and the testis, respectively, and the dipeptide amides are also predicted to cyclise to the corresponding DKP [30, 31]. It will be interesting to identify a function for Glu-Pro DKP and Phe-Pro DKP in their respective tissues.

In summary, we show that a cyclic dipeptide formed from TRH exhibits a potent activity in inhibiting the release of thyroglobulin from follicular thyroid cells, suggesting that these TRH-derived compounds may play a significant role in the inhibitory regulation of the thyroid axis. This inhibitory process taking place directly at the target site of TRH in the thyroid would be rapid and, in this respect, complements the known central mechanism for regulation of thyroid activity by providing an acute, rapid mechanism for fine tuning hormone production compared to the slow response of the negative feedback loop in the axis. These data, together with previous observations that a series of tripeptides with structures related to TRH occur in endocrine tissues and can give rise to DKPs, suggest that the dynamic control of the principal activity of a peptide by the opposing activity of its C-terminal peptide is a widespread and underappreciated aspect of peptide hormone biology.

Declarations

Compliance with ethical standards

Conflict of Interest: The authors declare there is no conflict of interest that could be perceived as prejudicing the impartiality of this article.

Ethical Approval: not required

Informed Consent: not required

Author contributions

DGSconceived the idea, DS, EG, MK, MG, PJK and DS[1] conceived and planned the presented experiments, DS, EG, CST, UR, LK, DWPC, HSA, MG and PJK performed the experiments, DGS and PJK wrote the

manuscript and all authors provided critical feedback.

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References

1. Jackson IM, Reichlin S (1979) Thyrotropin-releasing hormone in the blood of the frog, Rana pipiens: its nature and possible derivation from regional locations in the skin. Endocrinology 104:1814-1821. 10.1210/endo-104-6-1814
2. Yanagisawa T, Prasad C, Peterkofsky A (1980) The subcellular and organ distribution and natural form of histidyl-proline diketopiperazine in rat brain determined by a specific radioimmunoassay. J Biol Chem 255:10290-10294.
3. Melmed S, Carlson HE, Hershman JM (1982) Histidyl-proline diketopiperazine suppresses prolactin secretion in human pituitary tumour cell cultures. Clin Endocrinol (Oxf) 16:97-100. 10.1111/j.1365-2265.1982.tb03152.x
4. Fragner P, Presset O, Bernad N, Martinez J, Roze C, Aratan-Spire S (1997) A new biological contribution of cyclo(His-Pro) to the peripheral inhibition of pancreatic secretion. Am J Physiol 273:E1127-1132. 10.1152/ajpendo.1997.273.6.E1127
5. Smyth DG, del Rio-Garcia J, Wallnofer H, Gogl H, Simma W, Huber A, Embacher R, Fraser H, Kreil G (1999) Protirelin (thyrotropin-releasing hormone) in thyroid gland: possible involvement in regulation of thyroid status. Zhongguo Yao Li Xue Bao 20:289-291.
6. Gkonos PJ, Tavianini MA, Liu CC, Roos BA (1989) Thyrotropin-releasing hormone gene expression in normal thyroid parafollicular cells. Mol Endocrinol 3:2101-2109. 10.1210/mend-3-12-2101
7. De Miguel M, Fernandez-Santos JM, Utrilla JC, Carrillo-Vico A, Borrero J, Conde E, Guerrero JM, Martin-Lacave I (2005) Thyrotropin-releasing hormone receptor expression in thyroid follicular cells: a new paracrine role of C-cells? Histol Histopathol 20:713-718. 10.14670/HH-20.713
8. Castaldo C, Ciambellotti S, de Pablo-Latorre R, Lalli D, Porcari V, Turano P (2013) Soluble variants of human recombinant glutaminyl cyclase. PLoS One 8:e71657. 10.1371/journal.pone.0071657
9. Carotenuto D, Luchinat C, Marcon G, Rosato A, Turano P (2015) The Da Vinci European BioBank: A Metabolomics-Driven Infrastructure. J Pers Med 5:107-119. 10.3390/jpm5020107

10. Vlasov P, Doi SQ, Sellitti DF (2016) FRTL-5 Rat Thyroid Cells Release Thyroglobulin Sequestered in Exosomes: A Possible Novel Mechanism for Thyroglobulin Processing in the Thyroid. J Thyroid Res 2016:9276402. 10.1155/2016/9276402
11. Cummins PM, O'Connor B (1996) Bovine brain pyroglutamyl aminopeptidase (type-1): purification and characterisation of a neuropeptide-inactivating peptidase. Int J Biochem Cell Biol 28:883-893. 10.1016/1357-2725(96)00034-9
12. Fischer WH, Spiess J (1987) Identification of a mammalian glutaminyl cyclase converting glutaminyl into pyroglutamyl peptides. Proc Natl Acad Sci U S A 84:3628-3632. 10.1073/pnas.84.11.3628
13. Kimura T, Van Keymeulen A, Golstein J, Fusco A, Dumont JE, Roger PP (2001) Regulation of thyroid cell proliferation by TSH and other factors: a critical evaluation of in vitro models. Endocr Rev 22:631-656. 10.1210/edrv.22.5.0444
14. Schally AV, Redding TW, Bowers CY, Barrett JF (1969) Isolation and properties of porcine thyrotropin-releasing hormone. J Biol Chem 244:4077-4088.
15. Morley JE (1979) Extrahypothalamic thyrotropin releasing hormone (TRH) -- its distribution and its functions. Life Sci 25:1539-1550. 10.1016/0024-3205(79)90435-1
16. Fagerberg L, Hallstrom BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpoor S, Danielsson A, Edlund K, Asplund A, Sjostedt E, Lundberg E, Szigyarto CA, Skogs M, Takanen JO, Berling H, Tegel H, Mulder J, Nilsson P, Schwenk JM, Lindskog C, Danielsson F, Mardinoglu A, Sivertsson A, von Feilitzen K, Forsberg M, Zwahlen M, Olsson I, Navani S, Huss M, Nielsen J, Ponten F, Uhlen M (2014) Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics 13:397-406. 10.1074/mcp.M113.035600
17. Delbeke D, Van Sande J, Cochaux P, Decoster C, Dumont JE (1983) Effect of thyrotropin-releasing hormone on dog thyroid in vitro. Biochim Biophys Acta 761:262-268. 10.1016/0304-4165(83)90075-2
18. Iversen E, Laurberg P (1985) Thyrotrophin-releasing hormone (TRH) and hormone secretion from the follicular and C-cells of perfused dog thyroid lobes. Acta Endocrinol (Copenh) 109:499-504. 10.1530/acta.0.1090499
19. de Wied D, Gaffori O, Burbach JP, Kovacs GL, van Ree JM (1987) Structure activity relationship studies with C-terminal fragments of vasopressin and oxytocin on avoidance behaviors of rats. J Pharmacol Exp Ther 241:268-274.
20. Celis ME, Taleisnik S, Walter R (1971) Regulation of formation and proposed structure of the factor inhibiting the release of melanocyte-stimulating hormone. Proc Natl Acad Sci U S A 68:1428-1433. 10.1073/pnas.68.7.1428
21. Hiltz ME, Lipton JM (1989) Antiinflammatory activity of a COOH-terminal fragment of the neuropeptide alpha-MSH. FASEB J 3:2282-2284.
22. Huang KF, Liu YL, Cheng WJ, Ko TP, Wang AH (2005) Crystal structures of human glutaminyl cyclase, an enzyme responsible for protein N-terminal pyroglutamate formation. Proc Natl Acad Sci U S A

102:13117-13122. 10.1073/pnas.0504184102

23. Parish DC, Smyth DG, Normanton JR, Wolstencroft JH (1983) Glycyl glutamine, an inhibitory neuropeptide derived from beta-endorphin. Nature 306:267-270. 10.1038/306267a0
24. Rausell V, Fraser HM, Tobaruela M, del Rio-Garcia J, Smyth DG (1999) Identification of the TRH-like peptides pGlu-Glu-Pro amide and pGlu-Phe-Pro amide in rat thyroid: regulation by thyroid status. Regul Pept 81:55-60. 10.1016/s0167-0115(99)00017-8
25. Hirose Y, Ogawa N, Mori A (1985) Properties of histidyl-proline diketopiperazine [cyclo(His-Pro)] binding sites in rat liver. Regul Pept 12:279-288. 10.1016/0167-0115(85)90171-5
26. Cockle SM, Aitken A, Beg F, Smyth DG (1989) A novel peptide, pyroglutamylglutamylproline amide, in the rabbit prostate complex, structurally related to thyrotrophin-releasing hormone. J Biol Chem 264:7788-7791.
27. Bilek R, Gkonos PJ, Tavianini MA, Smyth DG, Roos BA (1992) The thyrotrophin-releasing hormone (TRH)-like peptides in rat prostate are not formed by expression of the TRH gene but are suppressed by thyroid hormone. J Endocrinol 132:177-184. 10.1677/joe.0.1320177
28. Khan Z, Aitken A, Garcia JR, Smyth DG (1992) Isolation and identification of two neutral thyrotropin releasing hormone-like peptides, pyroglutamylphenylalanineproline amide and pyroglutamylglutamineproline amide, from human seminal fluid. J Biol Chem 267:7464-7469.
29. Ghilchik MW, Tobaruela M, del Rio-Garcia J, Smyth DG (2000) Characterization of neutral TRH-like peptides in mammary gland, mammary tumors and milk. Biochim Biophys Acta 1475:55-60. 10.1016/s0304-4165(00)00043-x
30. Linden H, del Rio Garcia J, Huber A, Kreil G, Smyth D (1996) The TRH-like peptides in rabbit testis are different from the TRH-like peptide in the prostate. FEBS Lett 379:11-14. 10.1016/0014-5793(95)01468-3
31. Prasad C (1995) Bioactive cyclic dipeptides. Peptides 16:151-164. 10.1016/0196-9781(94)00017-z

Supplemental Figure

Supplemental Figure 1 is not available with this version

Figures

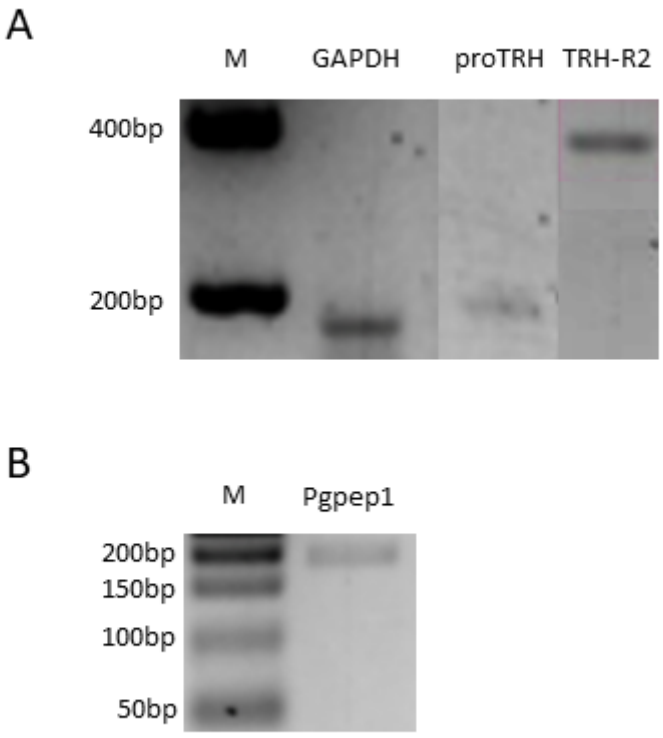

Figure 1

Legend not included with this version.

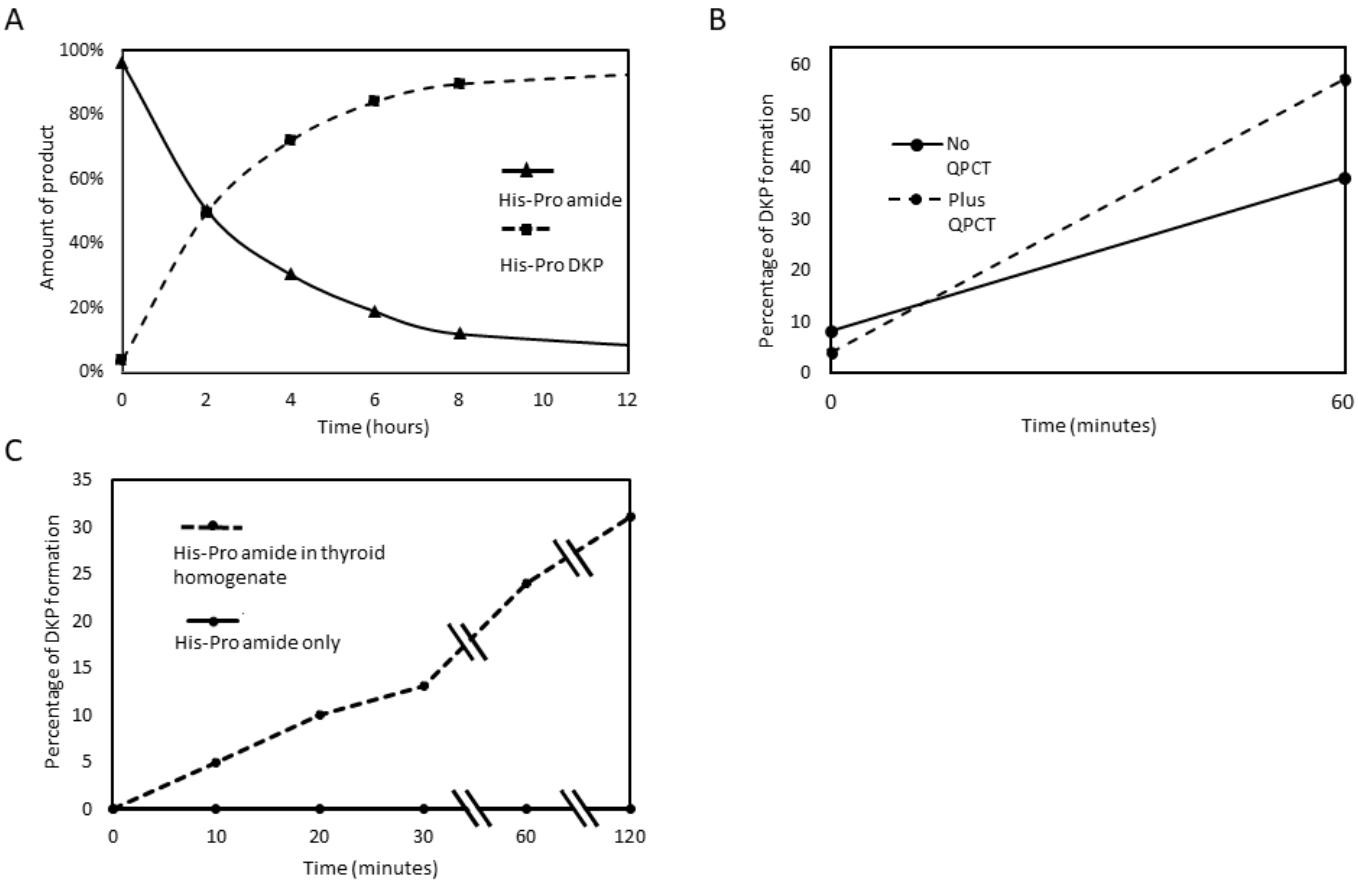

Figure 2

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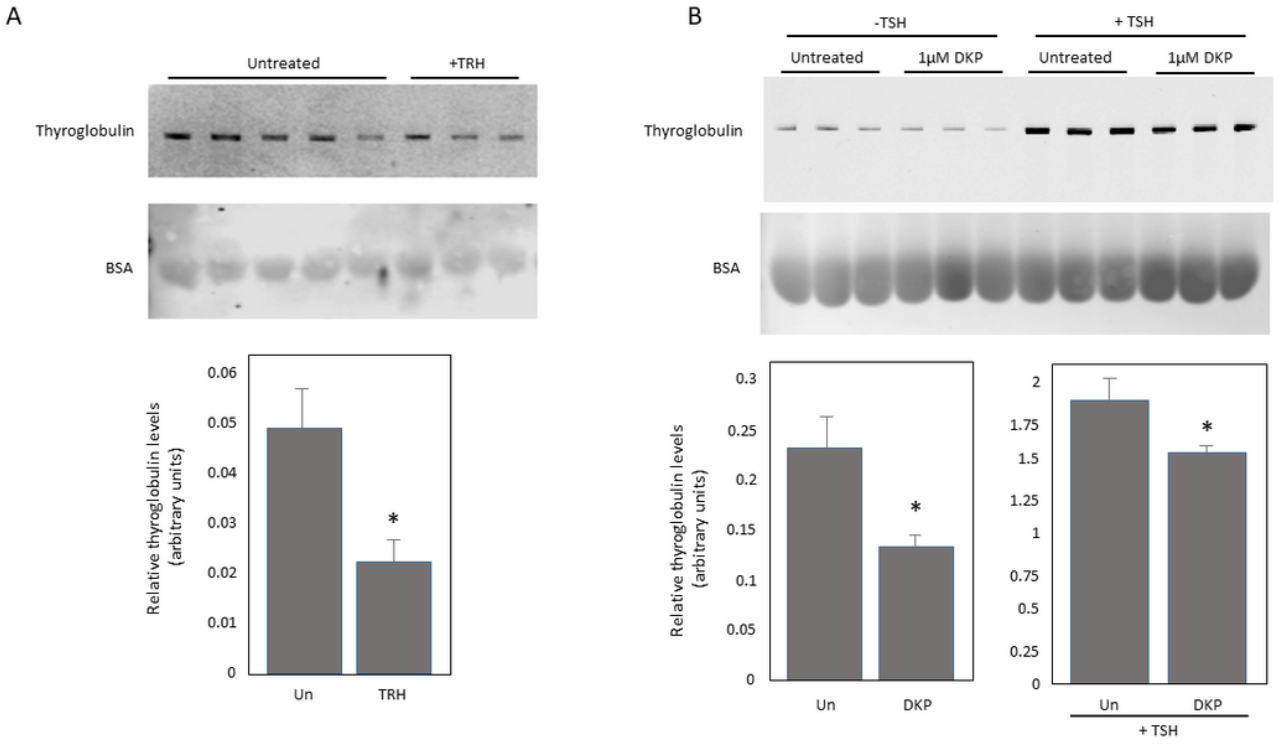

Figure 3

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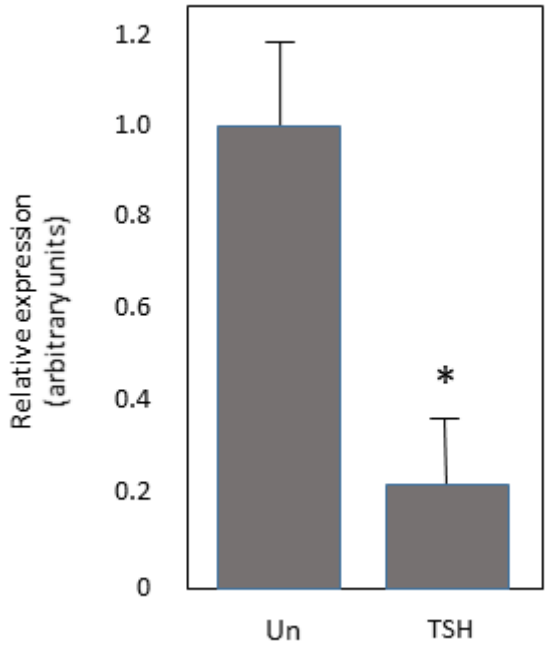

Figure 4

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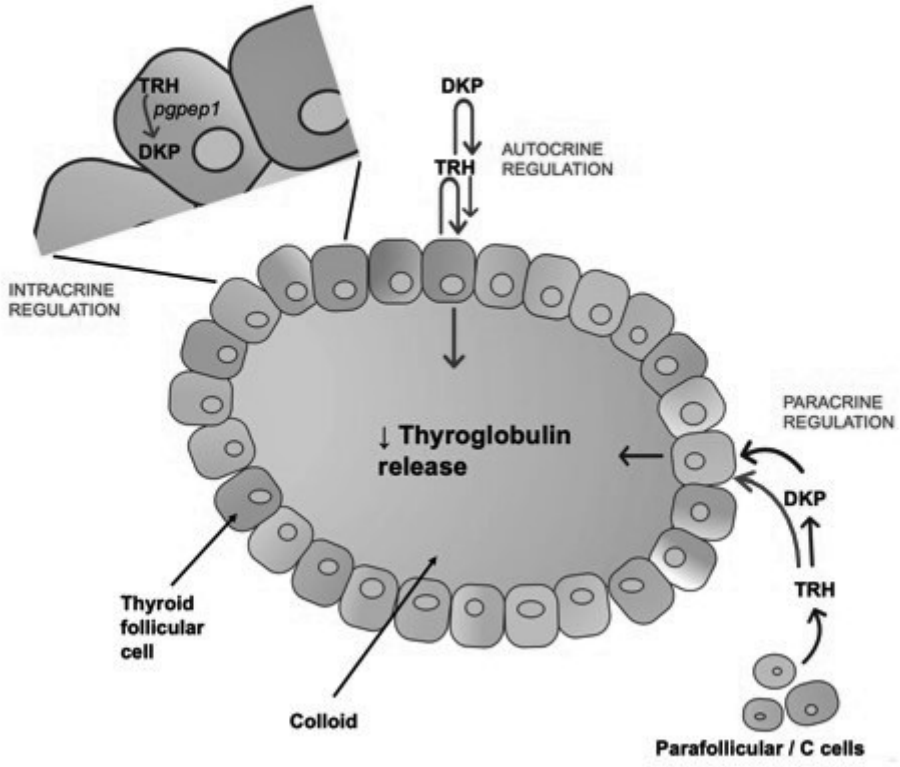

Figure 5

Legend not included with this version.