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A novel TRH analog, Gln-Asn-Pro-D-Tyr-D-TrpNH₂, binds to [³H][3-Me-His²]TRH-labelled sites in rat hippocampus and cortex but not pituitary or heterologous cells expressing TRHR1 or TRHR2

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Abstract

Gln-Asn-Pro-D-Tyr-D-TrpNH₂ is a novel synthetic peptide that mimics and amplifies central actions of thyrotropin-releasing hormone (TRH) in rat without releasing TSH. The aim of this study was to compare the binding properties of this pentapeptide and its all-L counterpart (Gln-Asn-Pro-Tyr-TrpNH₂) to TRH receptors in native rat brain tissue and cells expressing the two TRH receptor subtypes identified in rat to date, namely TRHR1 and TRHR2. Radioligand binding studies were carried out using [³H][3-Me-His²]TRH to label receptors in hippocampal, cortical and pituitary tissue, GH4 pituitary cells, as well as CHO cells expressing TRHR1 and/or TRHR2. In situ hybridization studies suggest that cortex expresses primarily TRHR2 mRNA, hippocampus primarily TRHR1 mRNA and pituitary exclusively TRHR1 mRNA. Competition experiments showed [3-Me-His²]TRH potently displaced [³H][3-Me-His²]TRH binding from all tissues/cells investigated. Gln-Asn-Pro-D-Tyr-D-TrpNH₂ in concentrations up to 10⁻⁵ M did not displace [³H][3-Me-His²]TRH binding to membranes derived from GH4 cells or CHO-TRHR1 cells, consistent with its lack of binding to pituitary membranes and TSH-releasing activity. Similar results were obtained for the corresponding all-L peptide. In contrast, both pentapeptides displaced binding from rat hippocampal membranes (pIC₅₀ Gln-Asn-Pro-D-Tyr-D-TrpNH₂: 7.7±0.2; pIC₅₀ Gln-Asn-Pro-Tyr-TrpNH₂: 6.6±0.2), analogous to cortical membranes (pIC₅₀ Gln-Asn-Pro-D-Tyr-D-TrpNH₂: 7.8±0.2; pIC₅₀ Gln-Asn-Pro-Tyr-TrpNH₂: 6.6±0.2). Neither peptide, however, displaced [³H][3-Me-His²]TRH binding to CHO-TRHR2. Thus, this study reveals for the first time significant differences in the binding properties of native and heterologously expressed TRH receptors. Also, the results raise the possibility that Gln-Asn-Pro-D-Tyr-D-TrpNH₂ is not displacing [³H][3-Me-His²]TRH from a known TRH receptor in rat cortex, but rather a hitherto unidentified TRH receptor.

Keywords

Thyrotropin-releasing hormone; TRH receptors; [³H][3-Me-His²]TRH binding; TRHR1; TRHR2; Gln-Asn-Pro-D-Tyr-D-TrpNH₂

G-protein-coupled receptors (GPCRs) are recognized to be involved in mediating the biological actions of neuropeptides and are viewed as attractive neuropharmacological targets [6]. To date, two GPCR subtypes have been identified in mammals for thyrotropin-releasing hormone (TRH): TRH receptor 1 (TRHR1) and TRH receptor 2 (TRHR2) [2,10,21]. In addition, a third receptor subtype has been cloned in *Xenopus laevis* (*xTRHR3*) [1]. Comparison of amino acid sequences of TRHR1 and TRHR2 from the same species shows that they have an overall homology of around 50% [22]. Human TRHR1 is around 90% homologous with mouse and rat TRHR1 at the cDNA and amino acid level [22]. TRHR2, however, has not been detected in humans [4]. In the rat, the distribution patterns of TRHR1 and TRHR2 are quite distinct, for example: TRHR1 is expressed at high levels in the pituitary and displays limited expression in the central nervous system (CNS), whereas TRHR2 is absent or present only at low levels in the pituitary and is widely distributed throughout the CNS [2,8,16,22]. The distinct regional distribution of TRHR1 and TRHR2 mRNA, supports the notion that TRHR1 plays a principal role in mediating the endocrine functions of TRH, while TRHR2 is important in mediating the higher cognitive functions of TRH, as well as its effects on arousal, locomotor activity and pain perception [8,22].

[³H][3-Me-His²]TRH is typically employed to label high affinity TRH receptor sites in radioligand binding studies in vitro, since it binds with greater affinity and affords higher specific binding than [³H]TRH [20]. Both TRHR1 and TRHR2 display similar high affinity for [³H][3-Me-His²]TRH and so this ligand cannot be used to discriminate between these two known TRH receptor subtypes [2,14]. Consistent with others, we have previously shown that [³H][3-Me-His²]TRH binds to a single population of high affinity sites in rat brain cortical membranes with a *K_d* of 4.54 nM [14]. Since in situ hybridization studies have revealed rat brain cortex predominantly expresses TRHR2, it could be speculated that the sites labelled by [³H][3-Me-His²]TRH in this tissue correspond to TRHR2, although it is not inconceivable that [³H][3-Me-His²]TRH is binding to a TRH receptor subtype in rat cortex that has yet to be identified.

We recently reported the discovery of a first in class peptide that is effective in producing and potentiating well-defined central TRH actions in rat without eliciting the release of TSH [18]. We demonstrated that this peptide, Glp-Asn-Pro-D-Tyr-D-TrpNH₂, displays a unique ability to not only potently inhibit the key enzyme degrading extracellular TRH, but also selectively bind to native central TRH receptors in rat brain cortex with unparalleled affinity. In particular, this peptide was found to bind to [³H][3-Me-His²]TRH-labelled receptors in rat brain cortex with a *K_i* value in the low nanomolar range, below that of [3-Me-His²]TRH. Prior to this, [3-Me-His²]TRH was recognized to be the only analog to bind to central TRH receptors with greater affinity than TRH [14,22]. Furthermore, it was found that the novel pentapeptide did not displace [³H][3-Me-His²]TRH binding to rat pituitary tissue, consistent with its lack of activity in stimulating TSH release.

The aim of the present study was to investigate the receptor binding properties of Glp-Asn-Pro-D-Tyr-D-TrpNH₂ and its all-L counterpart (Glp-Asn-Pro-Tyr-TrpNH₂) in native tissue from rat hippocampus and a variety of cells lines expressing TRHR1 and/or TRHR2 and to compare these to binding properties observed for rat cortex and pituitary. Cells that selectively express a specific receptor subtype are often used to study ligand-receptor interactions and signaling and have previously been employed to examine the binding characteristics of TRH analogs [5,9,16,13]. Specifically, the current study was undertaken by carrying out competitive radioligand binding assays in which [³H][3-Me-His²]TRH was employed to label TRH receptors. Comparison was made between binding in a GH4 pituitary cell line, which naturally expresses TRHR1, Chinese Hamster Ovary (CHO) cells transfected with TRHR1 or TRHR2 and native rat brain cortical, hippocampal and pituitary tissues. Since rat cortex is thought to express primarily TRHR2, the corresponding model

system employed was CHO cells stably transfected with TRHR2. Similarly, as rat hippocampus is indicated to express primarily TRHR1 and rat pituitary to express exclusively TRHR1, binding in these tissues was compared to binding to CHO cells stably transfected with TRHR1, as well as binding to the pituitary GH4 cell line. Unlabelled [3-Me-His²]TRH was used as a standard in all these competition binding experiments. Since bioluminescence resonance energy transfer studies indicate that TRHR1 and TRHR2 form heterodimers in cells [7], we also tested the binding of Glp-Asn-Pro-D-Tyr-D-TrpNH₂ in CHO cells co-expressing both receptors.

Glp-Asn-Pro-D-Tyr-D-TrpNH₂ and its all-L counterpart were custom synthesized by American Peptide Company (Sunnyvale CA). [³H][3-Me-His²]TRH (NET705, 58–72 Ci/mmol) was purchased from NEN Life Science Products.

Membranes were prepared from rat brain homogenates, GH4 pituitary cells or CHO cells transfected with cDNAs encoding either rat TRHR1 or TRHR2 as described previously [11,14]. The affinities of unlabelled compounds for TRH receptor binding sites in each preparation were determined in competition binding assays using [³H][3-Me-His²]TRH as radioligand. Briefly, all experiments were carried out in ice-cold sodium phosphate buffer containing 177 μ M bacitracin and 0.1% bovine serum albumin. Fifty microlitre aliquots of membrane suspensions were incubated with 6–8 nM [³H][3-Me-His²]TRH and increasing concentration of unlabelled competitor in a final volume of 100 μ l for 5 h at 4 °C. Non-specific binding was determined in the presence of 10 μ M TRH. All peptides employed in competition binding experiments were initially dissolved in DMSO at 10⁻² M and diluted to the desired concentration in assay buffer. The final concentration of DMSO did not exceed 1%, which did not affect the binding of [³H][3-Me-His²]TRH. Saturation binding experiments were carried out in the same way except that 50 μ l membrane suspensions were incubated with increasing concentrations of [³H][3-Me-His²]TRH (0.625–20 nM) in the absence (total binding) and presence (non-specific binding) of 10 μ M TRH. HPLC analysis [14,18] indicated that there is no evidence to suggest that TRH or TRH analogs undergo breakdown under the binding assay conditions employed. The incubation period was terminated by vacuum filtration through Whatman GF/B filters pre-soaked with sodium phosphate buffer. A 24-well Brandel cell harvester was used for this purpose. The filters were washed three times with ice-cold 0.9% sodium chloride solution before being placed in glass vials with 10 ml of water-miscible scintillation fluid (Ecoscint A, National Diagnostics). The radioactivity was allowed to disperse in the scintillation fluid overnight before counting in a Tri Carb 2900TR Liquid Scintillation Analyser. Radioligand binding results were analyzed using the non-linear curve fitting software GraphPad Prism 4.0. Data are represented as mean $\pm$ S.E.M. of *n* independent experiments.

The binding of [³H][3-Me-His²]TRH to native rat hippocampal and cortical membranes was measured in saturation binding experiments using 0.625–20 nM [³H][3-Me-His²]TRH. Specific binding was saturable and consistent with binding to a single population of sites in both cases. In the hippocampus, [³H][3-Me-His²]TRH bound with a *K_d* of 7.74 $\pm$ 1.65 nM with a *B_{max}* of 1.8 $\pm$ 0.05 fmol/mg wet weight (*n* = 4); for cortical membranes the apparent dissociation constant (*K_d*) was 5.3 $\pm$ 0.8 nM and the receptor density (*B_{max}*) was 3.3 $\pm$ 0.4 fmol/mg wet weight (*n* = 4).

Unlabelled [3-Me-His²]TRH displaced [³H][3-Me-His²]TRH from all native tissues and singly transfected cells tested with IC₅₀ values of 10⁻⁸–10⁻⁷ M (Fig. 1, Table 1). Glp-Asn-Pro-D-Tyr-D-TrpNH₂ displaced binding from hippocampus in a manner similar to that previously reported for cortex [18]. Comparable results were obtained for its all-L counterpart (Table 1). The D form of this pentapeptide had higher affinity than the L, and was 5–10-fold more potent than [3-Me-His²]TRH (Table 1). Neither Glp-Asn-Pro-D-Tyr-D-

TrpNH₂ nor its all-L counterpart displaced binding from CHO-TRHR1, CHO-TRHR2 or GH4 membranes in concentrations up to 10⁻⁵ M (Fig. 1, Table 1). TRH was found to displace [³H][3-Me-His²]TRH binding to CHO cells expressing both TRHR1 and TRHR2 with an IC₅₀ of 1.2±0.2×10⁻⁸ M, however, Glp-Asn-Pro-D-Tyr-D-TrpNH₂ did not displace [³H][3-Me-His²]TRH binding in this model system.

The observed lack of affinity of Glp-Asn-Pro-D-Tyr-D-TrpNH₂ and its all-L counterpart for [³H][3-Me-His²]TRH-labelled sites in GH4 and CHO-TRHR1 cells is in agreement with results reported for native pituitary tissue [18]. Moreover, each result is entirely consistent with in vivo data that show Glp-Asn-Pro-D-Tyr-D-TrpNH₂ does not evoke the release of TSH in rat [18].

Notably, however, the results of this study reveal important differences between native TRH receptors in rat hippocampus and cortex and heterologous cells expressing TRHR1 and TRHR2. As mentioned earlier, in situ hybridization analysis indicates that rat cortical tissue predominantly expresses TRHR2 mRNA [8,16]. We previously reported that Glp-Asn-Pro-D-Tyr-D-TrpNH₂ displaces [³H][3-Me-His²]TRH binding in rat cortex [18]. Yet, data from the current investigation reveal that Glp-Asn-Pro-D-Tyr-D-TrpNH₂ did not displace [³H][3-Me-His²]TRH binding to membranes prepared from CHO-TRHR2 cells. This raises questions as to what Glp-Asn-Pro-D-Tyr-D-TrpNH₂ and [³H][3-Me-His²]TRH are binding to in rat cortical tissue and the nature of the mechanism underpinning the central TRH-like behavioral actions of Glp-Asn-Pro-D-Tyr-D-TrpNH₂ [18]. Results from the present study also show that this pentapeptide potentially displaces [³H][3-Me-His²]TRH binding in rat hippocampal membranes. Since in situ hybridization analysis indicates that TRHR1 mRNA is predominantly expressed in hippocampal tissue, it would be expected that the results of hippocampal binding studies would be similar to those obtained from GH4 and CHO-TRHR1 cell lines. These results are clearly paradoxical for two reasons. On the one hand, binding data for Glp-Asn-Pro-D-Tyr-D-TrpNH₂ reveals that the hippocampus most closely resembles the cortex rather than the pituitary. On the other hand, the sites labelled in the cortex and in CHO-TRHR2 cells appear to be different. It could be speculated that hippocampal tissue contains TRHR2 to which the pentapeptide is binding. Indeed, although in situ hybridization studies indicate TRHR1 is the predominant TRH receptor subtype expressed in hippocampal tissue, it is possibly not exclusively present in the membrane preparation used, since TRHR2 has been observed in the precommissural hippocampus, as well as the subiculum [8,16]. Even if this was the case, the discrepancy between native TRH receptors in cortical tissue and expressed TRHR2 remains.

Several possible explanations exist. The first possibility is that the opportunity to form receptor-receptor hetero-oligomers may be lacking in cells selectively expressing a single receptor subtype, which in turn may alter receptor pharmacology [6]. The mediation of ligand signaling through GPCRs was initially understood to involve monomeric receptors. However, this view has been revised recently with the recognition that these receptors form homo-oligomeric and hetero-oligomeric complexes that influence GPCR receptor functioning and have implications regarding drug design [6]. For example, opioid receptor pairings of μ and δ subtypes have reduced affinity for ligands that are specific for each subtype [6]. Also, in relation to this, it has been suggested that data gathered from studies using isolated receptors may be misleading since the possibility of GPCR homo-hetero oligomerisation, which may be essential for ligand-receptor interactions and/or signaling, may not be possible under such circumstances [6]. Notably, in the case of TRH receptors, constitutive and agonist-induced homo-oligomerisation has been demonstrated, as well as TRH receptor subtype hetero-oligomer formation [7,15,24]. Thus, it is possible that formation of TRH receptor heterocomplexes occurs in native tissue and this is not possible in the cell models expressing a single receptor subtype. The absence of TRH receptor

heterocomplexes in cells could, potentially, give rise to the difference observed between native tissue and cells transfected with TRHR2. Nonetheless, co-expression of TRHR1 and TRHR2 in CHO cells did not lead to Glp-Asn-Pro-D-Tyr-D-TrpNH₂ binding. We have no direct experimental evidence of oligomerisation in the CHO cells co-expressing TRHR1 and TRHR2; nevertheless, it has been demonstrated using BRET and confocal microscopy that constitutive receptor hetero-oligomerisation occurs between TRHR1 and TRHR2 in cells expressing both receptor subtypes [7].

Secondly, the ligand binding specificities of a number of class B GPCRs is altered by receptor activity-modifying proteins (RAMPs), which interact directly with the receptor. For example, when calcitonin receptors are expressed alone they bind to calcitonin, but when they are expressed with RAMPs they bind to amylin or CGRP [17,19]. Although no equivalent proteins have been identified for the class A TRH receptor, it is possible that proteins present only in restricted regions of the CNS confer high affinity for Glp-Asn-Pro-D-Tyr-D-TrpNH₂. Additionally, differences between TRH receptors expressed in native tissue and cells lines could be due to differences in post-translational modification. GPCR structure and function may be modified following translation and it has been suggested that structural and functional heterogeneity arising from this mechanism may produce differential effects in cells or tissues in response to the same stimulation [23].

Another explanation of our results lies in the possibility that Glp-Asn-Pro-D-Tyr-D-TrpNH₂ may not be displacing [³H][3-Me-His²]TRH from a known TRH receptor in rat brain, but a hitherto unidentified TRH receptor subtype that is present both in rat brain cortex and hippocampus. The possible existence of novel receptors mediating some of the neuronal actions of TRH-like peptides has been suggested previously [9,3]. Indeed, this possibility may offer an explanation for the mismatch of high TRH-DE expression relative to TRH receptor expression observed in hippocampal tissue [6], as well as the occurrence of central effects of TRH in humans who appear to lack TRHR2. The existence of different receptor subtypes provides a means to achieve diversity in biological responses to a particular ligand; however, the functional significance that the potential multiplicity of TRH receptor subtype-interactions may subserve has yet to be elucidated. Subtype-selective analogs would be useful for probing the biological roles of TRH receptors. No such analogs had been identified until the recent publication of papers describing analogs that display reduced binding affinity in comparison to TRH, but a degree of selectivity for TRHR2 [13,12]. Interestingly, Engel et al. [5] recently reported several TRH analogs that displayed low binding affinity to cells stably expressing TRHR1 and TRHR2, but enhanced agonist efficacy, which may also prove useful for probing TRH signaling. Notably, by virtue of its unique properties, Glp-Asn-Pro-D-Tyr-D-TrpNH₂ may provide a novel means to investigate TRH signaling pathways and shed new light on the mechanism of receptor-mediated TRH actions in the CNS.

In conclusion, this is the first study to demonstrate the existence of important differences between TRH receptors in native brain tissue and heterologous cells expressing TRHR1 and/or TRHR2. Competitive radioligand binding studies with the novel peptide Glp-Asn-Pro-D-Tyr-D-TrpNH₂ and its all-L counterpart, but not [3-Me-His²]TRH, reveal these differences. Significantly, the data presented herein open up critical issues with regard to the use of cell lines expressing a homo- or hetero- class of TRHR subtype in pharmacology and drug screening studies. It may be speculated that an additional receptor may possibly be involved in TRH behavioral responses, however this would require further investigation. Significantly, Glp-Asn-Pro-D-Tyr-D-TrpNH₂ may provide a valuable innovative research tool with which to gain further understanding of the mechanisms underlying central TRH actions.

References

- [1]. Bidaud I, Lory P, Nicolas P, Bulant M, Ladram A. Characterization and functional expression of cDNAs encoding thyrotropin-releasing hormone receptor from *Xenopus laevis*. *Eur. J. Biochem* 2002;269:4566–4576. [PubMed: 12230569]
- [2]. Cao J, O'Donnell D, Vu H, Payza K, Pou C, Godbout C, Jakob A, Pelletier M, Lembo P, Ahmad S, Walker P. Cloning and characterization of a cDNA encoding a novel subtype of rat thyrotropin-releasing hormone receptor. *J. Biol. Chem* 1998;273:32281–32287. [PubMed: 9822707]
- [3]. Colson AO, Gershengorn MC. Thyrotropin-releasing hormone analogs. *Mini Rev. Med. Chem* 2006;6:221–226. [PubMed: 16472189]
- [4]. Engel S, Gershengorn MC. Thyrotropin-releasing hormone and its receptors—a hypothesis for binding and receptor activation. *Pharmacol. Ther* 2007;113:410–419. [PubMed: 17123625]
- [5]. Engel S, Neumann S, Kaur N, Monga V, Jain R, Northup J, Gershengorn MC. Low affinity analogs of thyrotropin-releasing hormone are super-agonists. *J. Biol. Chem* 2006;281:13103–13109. [PubMed: 16551618]
- [6]. George SR, O'Dowd BF, Lee SP. G-protein-coupled receptor oligomerization and its potential for drug discovery. *Nat. Rev. Drug Discov* 2002;1:808–820. [PubMed: 12360258]
- [7]. Hanyaloglu AC, Seeber RM, Kohout TA, Lefkowitz RJ, Eidne KA. Homo- and hetero-oligomerization of thyrotropin-releasing hormone (TRH) receptor subtypes. Differential regulation of beta-arrestins 1 and 2. *J. Biol. Chem* 2002;277:50422–50430. [PubMed: 12393857]
- [8]. Heuer H, Schafer MK, O'Donnell D, Walker P, Bauer K. Expression of thyrotropin-releasing hormone receptor 2 (TRH-R2) in the central nervous system of rats. *J. Comp. Neurol* 2000;428:319–336. [PubMed: 11064370]
- [9]. Hinkle PM, Pekary AE, Senanayaki S, Sattin A. Role of TRH receptors as possible mediators of analeptic actions of TRH-like peptides. *Brain Res* 2002;935:59–64. [PubMed: 12062473]
- [10]. Itadani H, Nakamura T, Itoh J, Iwaasa H, Kanatani A, Borkowski J, Ihara M, Ohta M. Cloning and characterization of a new subtype of thyrotropin-releasing hormone receptors. *Biochem. Biophys. Res. Commun* 1998;250:68–71. [PubMed: 9735333]
- [11]. Jones BW, Hinkle PM. Beta-arrestin mediates desensitization and internalization but does not affect dephosphorylation of the thyrotropin-releasing hormone receptor. *J. Biol. Chem* 2005;280:38346–38354. [PubMed: 16183993]
- [12]. Kaur N, Lu X, Gershengorn MC, Jain R. Thyrotropin-releasing hormone (TRH) analogues that exhibit selectivity to TRH receptor subtype 2. *J. Med. Chem* 2005;22:6162–6165. [PubMed: 16162016]
- [13]. Kaur N, Monga V, Lu X, Gershengorn MC, Jain R. Modifications of the pyroglutamic acid and histidine residues in thyrotropin-releasing hormone (TRH) yield analogs with selectivity for TRH receptor type 2 over type 1. *Bioorg. Med. Chem* 2007;15:433–443. [PubMed: 17035026]
- [14]. Kelly JA, Slator GR, O'Boyle KM. Pharmacologically distinct binding sites in rat brain for [3H]thyrotropin-releasing hormone (TRH) and [3H][3-methyl-histidine(2)]TRH. *Biochem. Pharmacol* 2002;63:2197–2206. [PubMed: 12110379]
- [15]. Kroeger KM, Hanyaloglu AC, Seeber RM, Miles LE, Eidne KA. Constitutive and agonist-dependent homo-oligomerization of the thyrotropin-releasing hormone receptor. Detection in living cells using bioluminescence resonance energy transfer. *J. Biol. Chem* 2001;276:12736–12743. [PubMed: 11278883]
- [16]. O'Dowd BF, Lee DK, Huang W, Nguyen T, Cheng R, Liu Y, Wang B, Gershengorn MC, George SR. TRH-R2 exhibits similar binding and acute signaling but distinct regulation and anatomic distribution compared with TRH-R1. *Mol. Endocrinol* 2000;14:183–193. [PubMed: 10628757]
- [17]. Parameswaran N, Spielman WS. RAMPs: the past, present and future. *Trends Biochem. Sci* 2006;31:631–638. [PubMed: 17010614]
- [18]. Scalabrino GA, Hogan N, O'Boyle KM, Slator GR, Gregg DJ, Fitchett CM, Draper SM, Bennett GW, Hinkle PM, Bauer K, Williams CH, Tipton KF, Kelly JA. Discovery of a dual action first-in-class peptide that mimics and enhances CNS-mediated actions of thyrotropin-releasing hormone. *Neuropharmacology* 2007;52:1472–1481. [PubMed: 17418282]

- [19]. Sexton PM, Morfis M, Tilakaratne N, Hay DL, Udawela M, Christopoulos G, Christopoulos A. Complexing receptor pharmacology: modulation of family B G protein-coupled receptor function by RAMPs. *Ann. N. Y. Acad. Sci* 2006;1070:90–104. [PubMed: 16888151]
- [20]. Sharif NA. Quantitative autoradiography of TRH receptors in discrete brain regions of different mammalian species. *Ann. N. Y. Acad. Sci* 1989;553:147–175. [PubMed: 2541648]
- [21]. Straub RE, Frech GC, Joho RH, Gershengorn MC. Expression cloning of a cDNA encoding the mouse pituitary thyrotropin-releasing hormone receptor. *Proc. Natl. Acad. Sci. U.S.A* 1990;87:9514–9518. [PubMed: 2175902]
- [22]. Sun Y, Lu X, Gershengorn MC. Thyrotropin-releasing hormone receptors – similarities and differences. *J. Mol. Endocrinol* 2003;30:87–97. [PubMed: 12683933]
- [23]. Tanoue A, Koshimizu T, Tsujimoto G, Nakata H, Hirose S, Fukuzawa T, Abe J, Kurose H. Heterogeneity of G protein-coupled receptor generated by post-translational mechanisms and its clinical meanings. *Nippon Yakurigaku Zasshi* 2004;124:235–243. [PubMed: 15467257]
- [24]. Zhu CC, Cook LB, Hinkle PM. Dimerization and phosphorylation of thyrotropin-releasing hormone receptors are modulated by agonist stimulation. *J. Biol. Chem* 2002;277:28228–28237. [PubMed: 12023974]

Fig. 1.

Displacement of [^3H][3-Me-His 2]TRH binding in membrane preparations from rat brain (cortex, hippocampus, pituitary) and cells (CHO-TRHR1, CHO-TRHR2 and GH4). Competitive inhibition curves of [3-Me-His 2]TRH (●) and Glp-Asn-Pro-D-Tyr-D-TrpNH $_2$ (○) vs. [^3H][3-Me-His 2]TRH binding are shown; reported results for cortex and pituitary [18] are included for purposes of comparison.

Table 1Displacement of [³H][3-Me-His²]TRH by TRH analogs in native tissue and cell models

Membrane source	[3-Me-His ²]TRH pIC ₅₀	Glp-Asn-Pro-D-Tyr-D-TrpNH ₂ pIC ₅₀	Glp-Asn-Pro-Tyr-TrpNH ₂ pIC ₅₀
Rat cortex	6.80 ± 0.2	7.8 ± 0.2	6.58 ± 0.2
Rat hippocampus	7.19 ± 0.2	7.68 ± 0.2	6.64 ± 0.2
Rat pituitary	7.77 ± 0.2	<5	<5
GH4 cells	7.95	<4	<4
CHO-TRHR1	7.58	<4	<4
CHO-TRHR2	7.76	<4	<4

Data are the mean of $n = 2$ or mean ± S.E.M. of $n = 3-16$ independent experiments each conducted in triplicate. pIC₅₀ is the negative log of the IC₅₀ value.