G PROTEIN-COUPLED RECEPTOR SIGNALLING IN NEUROENDOCRINE SYSTEMS

Thyrotropin-releasing hormone receptors – similarities and differences

Y Sun, X Lu and M C Gershengorn
National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892–1818, USA

(Requests for offprints should be addressed to M C Gershengorn; Email: marving@intra.niddk.nih.gov)

Abstract

Thyrotropin-releasing hormone (TRH) initiates its effects by interacting with cell-surface membrane receptors. Two G protein-coupled receptors for TRH, TRH receptor type 1 (TRH-R1) and TRH receptor type 2 (TRH-R2), have been cloned from mammals. In this review, we compare TRH-R1 and TRH-R2 with regard to their tissue distribution, binding affinities for TRH and TRH analogs, basal and activated signaling activities and characteristics of internalization. TRH-R1 and TRH-R2 are distributed differently in the brain and peripheral tissues, but exhibit indistinguishable binding affinities for TRH and TRH analogs. Although they both can be stimulated by TRH to similar maximal signaling levels, TRH-R2 exhibits higher basal signaling activity and is more rapidly internalized than TRH-R1. These differences in signaling and internalization properties are probably important in the distinct parts that TRH-R1 and TRH-R2 may play in mammalian physiology.

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Introduction

Thyrotropin-releasing hormone (TRH) is a tripeptide that is synthesized in the hypothalamus and released into the hypothalamic–pituitary portal circulation to act on the pituitary, and is produced in many other tissues, especially within the nervous system, where it appears to act locally. Hormonal and neurotransmitter/neuromodulator functions of TRH have been widely studied. A review of the actions of TRH was published in 1999 (Nillni & Sevarino 1999). It appears that TRH initiates all of these effects by interacting with receptors on the surfaces of cells. These receptors belong to the G protein-coupled receptor (GPCR) superfamily. In 1990, the first TRH receptor (TRH-R), later named type 1 TRH-R (TRH-R1), was cloned from a mouse pituitary tumor cDNA library and then orthologous receptors were cloned from a number of different species, including rat (de la Pena et al. 1992, Zhao et al. 1992, Sellar et al. 1993), chicken (Sun et al. 1998), cow (Takata et al. 1998), Catostomus commersoni (Harder et al. 2001a) and humans (Duthie et al. 1993, Matre et al. 1993). The human TRH-R1 is 90·3% and 89·2% homologous to the mouse and rat receptors at the DNA level, respectively; the three receptors exhibit approximately 95% homology at the amino acid level. There are splice variants of TRH-R1 at the C-terminus for rat and mouse; however, functional differences between these isoforms or their relative levels of expression in tissues have not been determined. A review of the molecular and cellular biology of TRH-R1 was published in 1996 (Gershengorn & Osman 1996). In 1998, a second subtype of TRH-R (TRH-R2) was identified in rat (Cao et al. 1998, Itadani et al. 1998, O'Dowd et al. 2000), mouse (Harder et al. 2001b) and Catostomus commersoni (Harder et al. 2001b); TRH-R2 has not yet been described in humans. Amino acid
sequence alignments of the two types of TRH receptors from the same species reveal a 50% overall identity. Subsequently, comparisons of TRH-R1 and TRH-R2 have been made regarding their tissue distribution, TRH binding and activation, basal signaling activities, internalization properties and structure. Herein we review these findings that demonstrate the important similarities and differences between the two TRH-R subtypes.

**Distribution of TRH-Rs in the rat**

TRH-R1 and TRH-R2 exhibit marked differences in anatomic distribution as determined by immunohistochemistry, in situ hybridization and northern blot analysis. In general, the findings obtained with different methods of assessment of expression are in agreement, but there are some exceptions. For example, TRH-R1 is found in the pyramidal layer of the hippocampal formation and TRH-R2 is observed in the arcuate nucleus, periventricular hypothalamic nucleus, preoptic area and suprachiasmatic nucleus in immunohistochemical studies, whereas most of the in situ hybridization studies show no expression in these areas. It is possible that TRH-Rs are present on nerve termini in one brain region that could arise from cell bodies in another region; these receptors would be detected only by immunohistochemical studies.

The distribution pattern of TRH binding sites has also been studied in dissected tissues (Burt & Taylor 1980, Ogawa et al. 1981, Simasko & Horita 1982, Taylor & Burt 1982) or by using quantitative binding autoradiography (Manaker et al. 1985, Mantyh & Hunt 1985, Pazos et al. 1985, Sharif & Burt 1985, Sharif 1989). These findings are in good agreement with the distributions of TRH-R1 and TRH-R2 found by in situ hybridization, although differences in the levels of expression estimated by the different methods are found. For example, the dentate gyrus shows high TRH binding but low TRH-R mRNA levels, whereas the motor nuclei of the cranial nerves have high levels of TRH-R mRNA but low amounts of TRH binding. As described above, such discrepancies might be due to the differences in methodology.

The distribution of TRH-Rs in the rat brain is described in detail below and is summarized in Table 1.

**Rhinencephalon and telencephalon**

TRH-R2 mRNA is expressed at low levels in the olfactory bulb and is absent in the accessory olfactory bulb, where TRH-R1 mRNA is highly expressed (Calza et al. 1992, Zabavnik et al. 1993, Heuer et al. 2000).

TRH-R2 is widely expressed in the pyramidal neurons throughout the layers of cerebral cortex (Heuer et al. 2000). By contrast, TRH-R1 is restricted to a few neurons in the outer layers of the cerebral cortex (Heuer et al. 2000). Regional distribution of TRH-R1 has been localized in the perirhinal cortex, the piriform cortex and the endopiriform nucleus (Calza et al. 1992), and TRH-R2 in the primary somatosensory and motor areas, primary visual area and primary olfactory cortex (O’Dowd et al. 2000). The high levels of expression of TRH-R2 in the cerebral cortex, a brain structure that is poor in TRH-R1 mRNA, strongly suggests that TRH-R2 may mediate the modulatory role of TRH on higher sensory and cognitive functions (for review, see Horita 1998). In the septum-diagonal band, TRH-R2 concentrates in the central part of the medial septum, whereas TRH-R1 was more prevalent in the lateral (Heuer et al. 2000, Zabavnik et al. 1993) and medial nuclei (Zabavnik et al. 1993).

In the hippocampal formation, TRH-R2 was highly abundant in the precommissural hippocampus, but not in the pyramidal neurons (Heuer et al. 2000). Relatively high TRH-R1 expression was seen in the ventral dentate gyrus (Calza et al. 1992, Heuer et al. 2000). Both TRH-R1 (Calza et al. 1992, Heuer et al. 2000, O’Dowd et al. 2000) and TRH-R2 were detected in the subiculum (Heuer et al. 2000, O’Dowd et al. 2000). TRH-R1 has also been observed in the CA3 field of Ammon’s horn (Zabavnik et al. 1993).

In the amygdala, TRH-R1 is highly expressed in the corticomedial nuclear complex, including the amygdalohippocampal area (Calza et al. 1992, Zabavnik et al. 1993, Heuer et al. 2000), which receives afferents from the olfactory bulb, the hypothalamus, and visceral nuclei of the brainstem. These findings support the view that TRH-R1 is the receptor for mediating visceral effects of TRH at the level of the amygdala (for review, see Hokfelt et al. 1989). In contrast, TRH-R2 mRNA was mainly detected in the central and basolateral amygdaloid complex, which is known to receive strong cortical and thalamic input, suggesting that
TRH-R2 may be involved in the modulation of cognitive functions at the level of the amygdala. In the caudate-putamen and globus pallidus, the expression of TRH-Rs is low or undetectable (Calza et al. 1992, Heuer et al. 2000).

Diencephalon

Most hypothalamic areas and nuclei express TRH-R1 mRNA (Calza et al. 1992, Zabavnik et al. 1993, Heuer et al. 2000). Low to moderate levels of TRH-R2 mRNA are present in the lateral anterior hypothalamic nucleus, the lateral hypothalamic area, and the dorsal portion of the ventromedial nucleus (Heuer et al. 2000).

In the thalamus, TRH-R1 mRNA expression is limited, but TRH-R2 is widely expressed in thalamic and subthalamic regions (Calza et al. 1992, Heuer et al. 2000, O’Dowd et al. 2000), suggesting a role for TRH-R2 in mediating the

<table>
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<tr>
<th>Region</th>
<th>TRH-R1</th>
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<td>Rhinal cortex</td>
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<td>Mammillary nucleus</td>
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+, expression of mRNA.
antinociceptive activity of TRH. It has been reported that the analgesic potency of TRH against certain chemical stimuli is comparable to that of morphine (Boschi et al. 1983).

**Mesencephalon and brainstem**

TRH-R2 is the predominant subtype in the mesencephalon and brainstem, where it is widely expressed with particularly high levels in the medial geniculate, the pontine nuclei, the reticular formation and the spinal nucleus of the trigeminal tract (Heuer et al. 2000, O’Dowd et al. 2000). These brainstem regions control the processing of sensory information and regulate activity, consciousness and sleep. In this regard, a well-known effect of TRH is to reduce the sleeping time of animals under drug-induced narcosis and improve sleep disorders in an animal model of narcolepsy (for review, see Horita 1998). In contrast, expression of TRH-R1 is restricted to a few areas, including the superior colliculi, the brainstem motor nuclei, the motor nucleus of the vagus and the nucleus of the solitary tract (Calza et al. 1992, Zabavnik et al. 1993, Heuer et al. 2000), which function to control neuroendocrine and other vegetative functions.

**Cerebellum**

High levels of TRH-R2 mRNA are detected in the cerebellar cortex, especially in the granule cell layer, and in interneurons scattered throughout the molecular layer (Calza et al. 1992, Heuer et al. 2000). The complete lack of TRH-R1 mRNA in the cerebellum indicates that TRH-R2 is the subtype mediating the effects of TRH on cerebellar neurons. For example, TRH ameliorates the cerebellar disorder in mutant and chemically induced ataxic mice (Kinoshita et al. 1995), and improves function in the cerebellar form of spinocerebellar degeneration (Sobue et al. 1983).

**Spinal cord**

The alpha-motoneurons in the spinal cord express TRH-R1 mRNA (Calza et al. 1992, Heuer et al. 2000), whereas TRH-R2 is expressed in neurons of the corticospinal tract (Heuer et al. 2000). As TRH-R2 is expressed at all levels of the central motor pathway, it is likely that the central effects of TRH on locomotor activation and control of movement are mediated by TRH-R2.

**Pituitary**

High levels of TRH-R1 expression are found in the anterior pituitary, whereas TRH-R2 mRNA could not be detected in the pituitary (Cao et al. 1998, Heuer et al. 2000) or could be detected only at low levels (O’Dowd et al. 2000).

**Peripheral tissues**

TRH-R1 mRNA is found in the heart, spleen, liver, lung, skeletal muscle, kidney and testes by northern blot analysis (Cao et al. 1998), and in the stomach, small intestine, colon, adrenal medulla, testis and pancreas by immunohistochemistry (Mitsuma et al. 1995). In contrast, TRH-R2 has limited peripheral distribution; it is present in the retina, testis and gastrointestinal tract (Mitsuma et al. 1999).

**Summary**

TRH-R1 is highly expressed in the anterior pituitary and the neuroendocrine brain regions, the autonomic nervous system and the visceral brainstem regions. In contrast, TRH-R2 is highly expressed in brain areas that are important for the transmission of somatosensory signals and higher central nervous system (CNS) functions. The anatomical distributions of the TRH-Rs provide a basis for understanding some of the CNS effects of TRH already reported, and for the design of studies to elucidate the biological roles of TRH under various physiological and pathological conditions.

**Comparison of the binding properties of TRH-R1 and TRH-R2**

During the past 30 years, numerous studies have been performed to define the binding characteristics of TRH-Rs. It is noteworthy that, although numerous analogs have been tested, only one synthetic analog, [methylHis]TRH (pGlu-methylHis-ProNH2), has been found that binds with greater affinity than TRH. In the early studies, binding was performed to tissue, cell and membrane preparations in which endogenous TRH-Rs were present. Some investigators claimed to distinguish more than one type of TRH-R on the basis of purported differences in binding characteristics (Rinehart et al.
In our opinion, however, no conclusive evidence for more than one type of high-affinity TRH-R has been presented in studies of tissue/cells expressing endogenous receptors.

After the cloning of TRH-R1, these studies were complemented by experiments in transfected cells. The discovery of TRH-R2 led to the expectation that differences in binding characteristics would be found between these two receptor subtypes. In the initial presentation of the cloning of TRH-R2 from rat brain, Cao and colleagues (1998) reported that pGlu-His-Pro-Gly exhibited different affinities in binding to TRH-R1 and TRH-R2. However, we found no differences in the affinities of binding of pGlu-His-Pro-Gly or the amidated pGlu-His-Pro-Gly-NH₂ to the two isotypes of TRH-Rs (O’Dowd et al. 2000). Moreover, the binding affinities of TRH-R1 and TRH-R2 cloned from mouse (Harder et al. 2001a), rat (Cao et al. 1998, Itadani et al. 1998) and Catostomus commersoni (Harder et al. 2001a) for native TRH or the higher-affinity analog, MeTRH, were identical. In our initial study, we compared the binding of a series of analogs that were substituted in the first position (Pro-His-Pro-NH₂ and desaza¹-His-Pro-NH₂) (Perlman et al. 1997), second position (pGlu-Val-Pro-NH₂) (Yu & Hinkle 1998), and third position (pGlu-His-pyrrolidine) (Yu & Hinkle 1998). We also studied a pair of conformationally restricted analogs, αCH-TRH and βCH-TRH, that exhibited stereospecific differences in binding to TRH-R1 (Laakkonen et al. 1996) and the freely rotatable parent analog, CH-TRH (pGlu-cyclohexylAla-Pro-NH₂). All analogs tested bound to TRH-R1 and TRH-R2 with similar affinities. More recently, Hinkle and colleagues (2002) reported that there were no differences in the affinities of TRH-R1 and TRH-R2 for a series of analogs substituted at the second position, including [Val²]TRH, [Phe²]TRH and [Tyr²]TRH, which are found in high concentrations within rat brain regions associated with regulation of mood. In summary, up to the present time, TRH-R1 and TRH-R2 have been found to exhibit indistinguishable binding affinities for TRH and TRH analogs.

Basal and TRH-stimulated signaling

TRH-Rs are members of the rhodopsin/β-adrenergic receptor family (family 1) of the GPCR superfamily. As shown in Fig. 1, intracellular signal transduction is mediated primarily by coupling to G₁₁ proteins. TRH binding results in the activation of phosphoinositide-specific phospholipase C, which stimulates phosphatidylinositol 4,5-P₂ (PIP₂) hydrolysis to form inositol 1,4,5-triphosphate (InsP₃) and 1,2-diacylglycerol (DAG). These second messengers stimulate increases in intracellular calcium and activation of protein kinase C (PKC) (Hsieh & Martin 1992, Kiley et al. 1991). TRH-R activation also stimulates calcium/calmodulin-dependent protein kinase (PKC) (Hsieh & Martin 1992, Kiley et al. 1991). TRH-R activation also stimulates calcium/calmodulin-dependent protein kinase (Jefferson et al. 1991, Cui et al. 1994) and mitogen-activated protein kinase (MAPK) (Kanda et al. 1994, Ohmichi et al. 1994). The TRH-R has also been shown to couple to G₁₂, G₁₃, and to a G₁-like protein that does not activate adenyl cyclase (Gershengorn & Osman 1996). Furthermore, in the cerebellum, TRH produces a region-specific and dose-dependent increase in cGMP concentrations (Mailman et al. 1978, Nakayama & Nagai 1996).

Several transcription factors have been shown to have roles in mediating TRH-induced gene

Although TRH-R1 and TRH-R2 signal via the same pathways, these receptors show marked differences in basal signaling activities. In the absence of ligands, activation of AP-1, Elk-1, and CREB was greater in cells expressing TRH-R2 than in cells expressing TRH-R1, indicating that TRH-R2 has marked TRH-independent signaling activity (Wang & Gershengorn 1999, Sun & Gershengorn 2002). The basal activities of TRH-R1 and TRH-R2 are inhibited by mida-zolam, an inverse agonist of TRH-Rs (Wang & Gershengorn 1999). In contrast to the difference in basal signaling, the potencies and the maximal levels of TRH-stimulated PIP2 hydrolysis by basal signaling, the potencies and the maximal protein kinase (Je

G protein subunits to dissociate from each other and both Ga and Gβγ can then transduce signals via different cellular effectors. G protein-mediated signaling is regulated by the balance of GDP/GTP exchange and GTP hydrolysis, which is accelerated by GTPase-activating proteins (GAPs). One of three groups of proteins that exhibit GAP activity toward heterotrimeric G proteins is the regulator of G protein signaling (RGS) proteins, which are members of a family of more than 20 proteins that inhibit (or desensitize) GPCR coupling (Berman & Gilman 1998, De Vries et al. 2000, Dohlman & Thorner 1997). It also appears that RGS proteins are effectors for these G proteins (De Vries & Gist Farquhar 1999). RGS4 has been shown to interact with Gaq, leading to an increase in its GTPase activity, and thereby to dampen signaling by agonists, which interact with some receptors that couple to Gaq (Hepler et al. 1997, Huang et al. 1997). TRH-R1 and TRH-R2 appear to signal by coupling to a Gq subfamily member (Aragay et al. 1992, Hsieh & Martin 1992). We demonstrated that RGS4, but not RGS7, RGS9 or GTPase-activator inhibiting protein, inhibited TRH signaling by both TRH-Rs without affecting receptor binding or expression (Harder et al. 2001). Moreover, RGS4 was shown to inhibit basal signaling by TRH-R1 and TRH-R2. It is noteworthy that RGS4 is expressed at high levels within the brain, especially in the paraventricular and mammillary nuclei of the hypothalamus and the olfactory cortex (Gold et al. 1997), where both TRH-R1 and TRH-R2 are expressed. Thus RGS4 may be an important physiological regulator of stimulated TRH-R signaling and its effect on basal signaling may be especially important in regulating TRH-independent signaling within the brain.

**Internalization**

Desensitization of signaling by agonist-induced receptor internalization provides a mechanism for rapid regulation of the density of receptors at the cell surface. This mechanism contrasts with the relatively slow effects of changes in receptor expression mediated by changes in gene transcription, mRNA stability or translation. In this context, it is interesting to note that high rates of turnover of TRH-R1 have been described in various cell types (Hinkle & Kinsella 1982, Drmota et al. 1998, Yu &
Hinkle 1998, 1999). More recently, TRH-induced internalization of TRH-R2 has been shown to be more rapid than that of TRH-R1 (O’Dowd et al. 2000, Sun & Gershengorn 2002). As TRH-R2 is widely distributed in the brain, the rapid agonist-induced internalization of TRH-R2 may be functionally significant in the CNS.

Like many GPCRs, TRH-R/ligand complexes may be internalized via clathrin-coated vesicles (Ashworth et al. 1995). A fraction of the receptor population is targeted to lysosomes, whereas the remainder is recycled to the cell surface. The ligands may remain associated with the receptor to return to the cell surface or may dissociate intracellularly, and are degraded in lysosomes (Petrou & Tashjian 1995). Most GPCRs undergo internalization that is dependent on phosphorylation, and TRH-induced receptor phosphorylation has been demonstrated recently (Zhu et al. 2002). However, whether phosphorylation has a role in TRH-R internalization has not been determined. Several mutations in TRH-R1 have led to mutant receptors that are defective in internalization, including receptors with mutations in transmembrane helix (TMH)-2, the third intracellular loop, and a truncated receptor missing the carboxyl terminus (Ashworth et al. 1995, Nussenzveig et al. 1993a,b, Petrou et al. 1997). Coupling to G protein is not sufficient to cause internalization (Nussenzveig et al. 1993b). It is not clear, however, whether receptor–G protein coupling influences TRH-R1 internalization (Nussenzveig et al. 1993b, Petrou et al. 1997, Yu & Hinkle 1999, Buck et al. 2000). In a recent study, chimeras of TRH-R1 and TRH-R2 were constructed to determine the structural basis of the differences in TRH-induced receptor internalization. It was demonstrated that the rate of internalization of a given chimera was more dependent on the origin of the extracellular domains and transmembrane helices than on the origin of the intracellular domains of the receptors (Sun & Gershengorn 2002), even though it is the intracellular domains that are believed to interact with the proteins that mediate internalization. In fact, we concluded that the active conformation of the TRH receptor was more important for internalization than the specific amino acid sequences of the receptor. The concept that there are direct correlations between the active states and internalization rates has been proposed for other GPCRs (Min et al. 1998).

TRH-R1 and TRH-R2 are conformationally distinct – studies of a conserved tryptophan in transmembrane helix 6

Many studies have been performed in attempts to delineate the three-dimensional (3D) structure of GPCRs. Recently, the 3D structure of rhodopsin, a prototypical GPCR of the same family as TRH-R, was resolved by X-ray crystallography (Palczewski et al. 2000). Using computer simulations, we have constructed a 3D model of TRH-R1 based on the rhodopsin structure that agrees well with our previously reported models (Y Sun, X Lu & MC Gershengorn, unpublished observations). In the past, we had constructed models of TRH-R1 based on a generic structure for GPCRs that was in turn based on an analysis of GPCR sequences. The constructed model was computationally optimized using energy minimization and molecular dynamics simulations (Gershengorn & Osman 2001). Thereafter, the model was repeatedly tested and refined on the basis of experimental results. The models predict a TRH binding pocket within the TMH bundle of TRH-R1 and are consistent with the hypothesis that there are residues in the extracellular loops with which TRH may interact before entering the TMH pocket. Consistent with the indistinguishable binding properties of TRH-R1 and TRH-R2, the four residues in the TMHs that form the binding pocket and two residues in the extracellular loops that appear to be important for TRH recognition in TRH-R1 are conserved in TRH-R2. However, as described above, TRH-R1 and TRH-R2 exhibit markedly different basal activities (Wang & Gershengorn 1999, Sun & Gershengorn 2002). These findings per se suggest there are important differences in the conformations of the unoccupied TRH-Rs.

Many mutated GPCRs have been found to signal basally, and it has been proposed that activating mutations release the native receptor from a constrained, inactive conformation. Several groups have suggested that specific interactions between residues in the helical bundles are primarily involved in these constraining interactions (Robinson et al. 1992, Scheer et al. 1996, Groblewski et al. 1997, Lin et al. 1997). For TRH-R1, we showed that a Trp residue in TMH-6 was one such constraining residue, because
substitution by Ala yielded a mutant receptor that exhibited high basal activity even though TRH-R1 has only minimal basal activity (Colson et al. 1998). Our model for TRH-R1 predicted that the Trp in TMH-6, which is highly conserved in family 1 GPCRs, is part of a hydrophobic cluster composed of aromatic residues in TMH-5 and TMH-6 that hold these two helices in close proximity to each other (Gershengorn & Osman 1996). Molecular dynamics simulations of TRH-R1 without and with bound TRH demonstrated that the average distance between the intracellular portions of TMH-5 and TMH-6 increases after TRH binds to TRH-R1. The same analysis performed on the unoccupied Trp to Ala mutant receptor revealed a similar increase in the distance between TMH-5 and TMH-6. These findings were interpreted as showing that release of a constraint imposed by the Trp near the top of TMH-6 results in a conformational change transduced through the helices to intracellular loop-3, which was shown to be involved in coupling TRH-R1 to $G_{q/11}$ (Aragay et al. 1992, Hsieh & Martin 1992). This may be the basis for basal activation of the mutant receptor (Colson et al. 1998). In contrast to our findings with TRH-R1, mutation of the corresponding Trp to Ala in TMH-6 of TRH-R2 produced a receptor with no observable basal activity (Sun & Gershengorn 2002). (A 3D model of TRH-R2 has not yet been constructed.) Thus, it is clear that there are important differences in the 3D structures of TRH-R1 and TRH-R2 that account for the differences in their signaling properties.

**Conclusion**

Two types of TRH-Rs are present in several species, although TRH-R2 has not yet been found in humans. Rodent receptors exhibit identical affinities for TRH (no other natural ligands for TRH-Rs have been found) and appear to signal via the same transduction pathways. Why, then, are there two TRH-Rs? It is likely that there are important differences between the responses initiated by the two receptors, as they are expressed in distinct distributions. The major difference found so far is that these receptors assume different conformations, leading to differences in their signaling activities. As discussed above, the differences in internalization rates may also be a consequence of the receptor conformation that leads to signaling. Thus it may be that, in certain tissues, persistent signaling, independent of TRH, is needed and that TRH is needed to add only a small increase in signaling. In this circumstance, for example in certain regions of the cerebral cortex, TRH-R2 might be expressed. We wonder whether there may be natural inverse agonists that inhibit signaling by TRH-R2, as it has been shown that there is an agouti-related peptide that is an inverse agonist for the melanocortin-4 receptor (Haskell-Luevano & Monck 2001). In other tissues, for example within the pituitary, it is necessary for signaling to be more tightly controlled by TRH, and TRH-R1 is expressed. In order to understand more fully the physiology of the TRH/TRH-R system, these hypotheses must be tested, with particular emphasis on how the differences in signaling by TRH-R1 and TRH-R2 subserve distinct functions.

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