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News and reviews

Role of thyrotropin-releasing hormone in prolactin-producing cell models

Haruhiko Kanasaki *, Aki Oride, Tselmeg Mijiddorj, Satoru Kyo

Department of Obstetrics and Gynecology, Shimane University School of Medicine, Izumo 693-8501, Japan

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ABSTRACT

Thyrotropin-releasing hormone (TRH) is a hypothalamic hypophysiotropic neuropeptide that was named for its ability to stimulate the release of thyroid-stimulating hormone in mammals. It later became apparent that it exerts a number of species-dependent hypophysiotropic activities that regulate other pituitary hormones. TRH also regulates the synthesis and release of prolactin, although whether it is a physiological regulator of prolactin that remains unclear. Occupation of the Gq protein-coupled TRH receptor in the prolactin-producing lactotroph increases the turnover of inositol, which in turn activates the protein kinase C pathway and the release of Ca^{2+} from storage sites. TRH-induced signaling events also include the activation of extracellular signal-regulated kinase (ERK) and induction of MAP kinase phosphatase, an inactivator of activated ERK. TRH stimulates prolactin synthesis through the activation of ERK, whereas prolactin release occurs via elevation of intracellular Ca^{2+}. We have been investigating the role of TRH in a pituitary prolactin-producing cell model. Rat pituitary somatolactotroph GH3 cells, which produce and release both prolactin and growth hormone (GH), are widely used as a model for the study of prolactin- and GH-secreting cells. In this review, we describe the general action of TRH as a hypophysiotropic factor in vertebrates and focus on the role of TRH in prolactin synthesis using GH3 cells.

Contents

1. Introduction

Thyrotropin-releasing hormone (TRH) was initially isolated from ovine and porcine hypothalamic extracts due to its ability to stimulate the release of thyroid-stimulating hormone (TSH) from rat pituitary cells (Nair et al., 1970; Burgus et al., 1970). The TRH sequence (pGlu-His-Pro-NH_2) has been fully conserved from fish to mammals, but this peptide is known to exert diverse, species-specific effects on the pituitary gland. TRH is widely expressed in various brain regions and the spinal cord (Poulat et al., 1992; Merchenthaler et al., 1988), as well as in a number of peripheral organs, including the pituitary (Le Dafniet et al., 1990), thyroid (Iversen et al., 1984), placenta (Shambaugh et al., 1979), pancreas (Leduque et al., 1989) and testis (Montagne et al., 1996).

2. Effect of TRH on pituitary cells

Although TRH was originally named as a TSH-releasing hormone, there is now considerable evidence that TRH is involved in species-specific hypophysiotropic functions. The levels of TRH in several species

* Corresponding author at: Shimane University, School of Medicine, Dept. of Obstetrics and Gynecology, 89-1 Enya Cho, Izumo City 693-8501, Shimane Prefecture, Japan.
E-mail address: kanasaki@med.shimane-u.ac.jp (H. Kanasaki).

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of fish, such as carp, tilapia and African lungfish, are not capable of stimulating TSH (Kagabu et al., 1998; Melamed et al., 1995; Gorbman and Hyder, 1973). In other types of fish, TRH exerts a modest effect on TSH stimulation, and corticotropin-releasing hormone (CRH) seems to play the role of a TSH-releasing factor, at least in coho salmon (Larsen et al., 1998). Indeed, in amphibians, CRH appears to be the major TSH-stimulating factor (Okada et al., 2009). TRH is known to stimulate the secretion of growth hormone (GH) in carp (Kagabu et al., 1998) and is a potent stimulator of α-melanocyte-stimulating hormone in teleost fish (Tran et al., 1989). TRH is also a potent stimulator of prolactin secretion in amphibians (Castano et al., 1993) and is the major prolactin-releasing factor in the hypothalamus of the bullfrog (Nakajima et al., 1993). In the pituitary in mammals, TRH stimulates not only TSH, but also prolactin and GH (Szabo et al., 1984; Tashjian et al., 1971).

3. TRH as a prolactin-releasing factor

Shortly after its initial identification as a TSH-releasing factor, TRH was shown to cause the rapid release of prolactin from rat anterior pituitary cells (Tashjian et al., 1971). However, it is not clear whether TRH is a physiological regulator of prolactin. Indeed, a number of different experimental approaches have failed to clarify the physiological role of TRH. In humans, the same concentrations of TRH that release TSH also release prolactin (Noel et al., 1974). In rats, the neutralization of endogenous TRH with TRH antisera suppresses the basal secretion of prolactin (Koch et al., 1977), and suckling causes an increase in hypothalamic and portal TRH levels along with a decrease in dopamine levels (de Greef et al., 1981). In human hypothyroidism, basal levels of TSH and prolactin, as well as their response to TRH, are increased (Snyder et al., 1973). On the contrary, immunoneutralization of TRH did not affect the magnitude of prolactin release in proestrus rats (Horn et al., 1985). TRH knockout mice displayed hypothyroidism with elevated TSH but had normal prolactin levels (Yamada et al., 1997). However, observations of TSH levels during lactation and the evaluation of prolactin levels in various thyroid states suggest that TRH is a physiological prolactin-releasing factor, albeit not the primary factor or one of major importance.

4. Rat pituitary somatolactotroph GH3 cell line

Pituitary somatotrophs and lactotrophs are both acidophilic cells and are believed to derive from the same origin. In the anterior pituitary gland of normal adult rats, acidophilic cells are almost equally divided into somatotrophs, lactotrophs and somatolactotrophs (Frawley et al., 1985). Somatolactotrophs, however, appear earlier than somatotrophs and lactotrophs during neonatal development in rats, and the proportion of lactotrophs has been reported to be only 1.7% of all anterior pituitary cells (Hoeffler et al., 1985). These observations suggest the possibility that prolactin-secreting cells arise from GH-secreting cells. GH3 cells, established from rat pituitary adenoma, can synthesize and secrete both prolactin and GH, and exist as either somatotrophs or somatolactotrophs (Boockfor and Schwarz, 1988). Thus, GH3 cells are widely used as a model to study the functions of normal pituitary acidophilic cells.

5. Intracellular signaling evoked by TRH in GH3 cells

It is known that TRH stimulates production of inositol phospholipid by activating the Gq protein-coupled TRH receptor in lactotrophs. This in turn stimulates the protein kinase C pathway (PKC) and Ca^{2+} release from Ca^{2+} storage sites (Gershengorn, 1986). TRH-induced signaling activates the extracellular signal-regulated kinase (ERK) via PKC-dependent and PKC-independent pathways—the former pathway activates MEK kinase (MEKK) via PKA and the latter induces Ras-dependent MEKK activation via tyrosine phosphorylation of Shc proteins. MEKK activates MEK by phosphorylation and ultimately ERK is activated by MEK (Winitz et al., 1993; Ohmichi et al., 1994). TRH-induced ERK activation is inactivated via dephosphorylation by dual specificity protein phosphatases, MAP kinase phosphatases (MKPs) (Oride et al., 2009). On the other hand, the elevation of intracellular Ca^{2+} levels from Ca^{2+} storage sites, via the mobilization of inositol phospholipid or the influx of extracellular Ca^{2+} through calcium channels, activates Ca^{2+}-dependent protein kinases such as Ca^{2+}/calmodulin-dependent protein kinase II (Jefferson et al., 1991; Cui et al., 1994).

6. TRH signaling and prolactin synthesis/secretion

The TRH-induced release and synthesis of prolactin are regulated by different signaling cascades. The TRH-induced activation of ERK is strongly involved in prolactin gene expression, as shown by the expression of TRH-induced prolactin mRNA being completely inhibited in the presence of a MEK (an activator of ERK) inhibitor (Kanasaki et al., 1999, 2002). In addition, overexpression of the constitutively active form of MEKK increases the expression of endogenous prolactin mRNA (Kanasaki et al., 2002). These observations are explained by the fact that the prolactin promoter contains binding sites such as Ets-1 and GFH-1/Pit-1, both of which synergistically enhance the synthesis of prolactin in a Ras/Raf response element within the prolactin promoter that is related to ERK signaling pathways (Bradford et al., 1995). However, the release of TRH-stimulated prolactin does not occur via ERK activation. The MEK inhibitor did not inhibit the release of TRH-induced prolactin, but it did inhibit it in the presence of the inhibitor for Ca^{2+}/calmodulin-dependent protein kinase II or myosin light chain kinase, both of which are dependent upon Ca^{2+} for activation (Kanasaki et al., 1999, 2002). These observations clearly demonstrate that hormone release and synthesis are regulated differently by TRH stimulation.

In GH3 cells, both prolactin and GH are regulated by TRH stimulation; GH synthesis is reduced by TRH, whereas prolactin synthesis is stimulated (Kanasaki et al., 2002). Previous studies have demonstrated that treating GH3 cells with TRH results in an increased number of prolactin-producing cells and a decreased number of GH-secreting cells (Boockfor et al., 1985; Schonbrunn et al., 1980). Epidermal growth factor (EGF) has an effect similar to that of TRH as it increases prolactin and decreases GH levels in GH4Cl cells, a subclone of GH3 cells (Schonbrunn et al., 1980). TRH-induced ERK activation is strongly involved in this phenomenon as the inhibition of ERK does not increase prolactin synthesis but increases GH synthesis. In addition, overexpression of the constitutively active form of MEKK decreases GH synthesis (Kanasaki et al., 2002). If an increasing population of prolactin-producing cell was in the more differentiated stage among somatolactotrophs, it is possible that TRH could act as a differentiation-promoting factor, leading to a decrease in the proportion of somatotrophs and an increase in the proportion of lactotrophs. The proposed mechanisms of TRH action in hormone synthesis and release in GH3 cells is shown in Fig. 1.

7. Mode of TRH delivery and the effect on prolactin expression

It is well known that hypothalamic gonadotropin-releasing hormone (GnRH) is delivered into portal circulation in a pulsatile manner and its pulse frequency determines predominant output of pituitary gonadotropins, namely luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Crowley et al., 1985). The release of GnRH needs to be in a pulsatile manner, and not continuous, to maintain the secretion of gonadotropins (Knobil, 1980). In addition, a more rapid pulse frequency of GnRH predominantly increases the secretion of LH, whereas a slower pulse frequency of GnRH decreases LH secretion and increases FSH release (Wildt et al., 1981). As for TRH, its secretion was found to be episodic and irregular in the venous blood of the pituitary in mares (Alexander et al., 2004), whereas in humans TRH is released in a pulsatile manner with regard to frequency and amplitude (Adriaanse et al.,