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Thyrotropin-releasing hormone in cardiovascular pathophysiology

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Abstract

Thyrotropin (TSH)-releasing hormone (TRH) also known as thyroliberin was the first of a number of peptides exerting several roles as a hormone and as a neuropeptide. Its ubiquitous distribution in the hypothalamus and in the extrahypothalamic regions and its diverse pharmacological and physiological effects are all features of its dual functions. For this reason, TRH has been the subject of much research throughout the past 20 years, work that has examined the structure, function, distribution, and regulation of the tripeptide and it has been extensively reviewed elsewhere [1,2] [O'Leary R., O'Connor B. Thyrotropin-releasing hormone. J Neurochem. 1995;65:953–963.; Nillni E., Sevarino K. The biology of pro-thyrotropin-releasing hormone-derived peptides. Endocrine Reviews, 1999;20:599–664.].

After a brief overview of its distribution, hypothalamic and extrahypothalamic functions, and receptors involved, this review discusses efforts devoted to support TRH role in cardiovascular regulation with a main focus on hypertension pathophysiology in experimental models and humans.

Keywords: TRH; Thyroliberin; Antisense; iRNA; Blood pressure; Hypertension; Obesity; Leptin

1. Introduction

TRH from porcine hypothalamus was the first hypothalamic hypophysiotropic hormone to be purified and characterised [3,4]. TRH is a weakly basic tripeptide with the amino acid sequence pyroglutamyl-histidyl-proline amide (pGlu-His-Pro-NH2). This tripeptide has hard conformational requirements for receptor activation, and almost any alteration from the structure of native thyroliberin results in substantial or complete loss of biological activity [5].

1.1. The TRH gene and preproTRH structure

It has been shown that TRH, like other hypothalamic release-inducing factors, including lutenising hormone-releasing hormone (LHRH), arises from the posttranslational cleavage of a large precursor protein. The cDNA of the mammalian preprohormone of TRH (preproTRH) was first cloned by Lechan et al. [6]. The genomic organization of the rat-preproTRH gene is well known [7,8]. PreproTRH is the product of a single copy of the preproTRH gene in the rat and other species genomes. The gene is approximately 2.6 kb in size and contains three exons interrupted by two introns. Although the complete mechanism regulating the expression of this gene is incompletely understood, characteristics promoter elements in the 5′ region of the preproTRH gene include TATA and GC box sequences, sequences similar to cAMP response element (CRE), negative thyroid response elements (TREs), SP1-binding region and elements present in catecholamine and glucocorticoid, induced genes. PreproTRH gene promoter also includes an insulin tissue-specific enhancer sequence which can explain the high expression of this gene in neonatal pancreas [7].

The deduced amino acid sequence of the preproTRH reveals that it is 26 kDa in molecular mass composed of 255 amino acids and contains five copies of the TRH progenitor sequence Gln-His-Pro-Gly flanked by pairs of basic residues where specific processing enzymes produce

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their cleavage followed by the removal of the basic residue before amidation that is essential for the tripeptide bioactivity [5–8].

1.2. Tissue TRH distribution

The TRH precursor has been localized by immunohystochemical techniques in several hypothalamic nuclei, the medullary raphe, and regions of the telencephalon, including the diagonal band of Broca, medial and lateral septum, and bed nucleus of the stria teminalis, in a distribution identical to that reported for the mature tripeptide [9]. The availability of TRH in pure form led to the development of specific radioimmunoassays for the tripeptide and the realisation that TRH, identical to that found in the hypothalamus, also occurs in extrahypothalamic brain regions [10].

TRH-like immunoreactivity or biological activity is widely distributed not only throughout the central nervous system, brain, spinal cord but gastrointestinal tract, and the body fluids of several mammalian species [11,12].

Particularly TRH positive neurons have been described in the parvocellular division of the paraventricular nucleus (PVN) that project to the media eminence (ME). However, not all TRH-containing neurons in the PVN project to the ME [13]. In addition TRH neurons are present in many other regions of the hypothalamus (dorsomedial nucleous, lateral hypothalamus, preoptic area, periventricular nucleous) [14] and extrahypothalamic regions (diencephalon, telencephalon, mesencephalon and spinal cord) which do not innervate the ME and are not regulated as the thyrotrophic neurons of the PVN suggesting their involvement in the extrahypophysiotropic TRH actions.

1.3. TRH receptors

Although TRH receptors are out of the scope of the present review, briefly, full-length cDNA for the TRH receptor (TRH-R1) in mouse, rat and human have been cloned by different groups [15–17]. In 1998, a second subtype of TRH receptor was described and cloned (TRH-R2) being 50% homologous compared with the subtype 1 and it was found to be the predominant TRH receptor subtype in the central nervous system [18] which seems to modulate calcium influx after TRH binding.

The postreceptor activation mechanism for both subtypes involves the phospholipase *C*-based hydrolysis of inositol phospholipids, leading to Ca^{2+} and diacylglycerol-activated protein kinase action. It has been reported that tight coupling of TRH-Rs stimulated inositol trisphosphate formation in rat pituitary cells. TRH has also been reported to stimulate polyphosphoinositide hydrolysis by a guanine nucleotide-modulated mechanism. However, there have been reports that TRH possesses two distinct types of brain intracellular signalling systems, namely, a cyclic AMP- and an inositol phosphate-based system, which may vary with brain regions [19].

1.4. Extrahypothalamic TRH functions

In addition to its classical TSH-releasing action, TRH has also been implicated in the release of growth hormone in several endocrine, neuropsychiatric, and metabolic disorders, which may be due to a change in the balance of other factors regulating growth hormone release [20,21].

TRH may be considered as a neurotransmitter. In fact, its extrahypothalamic distribution in the brain, localization at the synaptic level, presence in secretory granules, release at synaptic terminals, binding to high-affinity receptors that show a remarkable degree of anatomical localisation, specific effects on neuronal activity, stimulation of a wide range of centrally mediated behavioral effects, and the presence of brain peptidases capable of inactivating the tripeptide provide a complete list of criteria consistent with such a neuronal function. Some central effects of TRH injected intracerebroventricular (icv) or applied to specific brain regions are as follows: 1—Reversal of sedation induced by narcotics or alcohol; 2—Reversal of natural sedation or hibernation; 3—Energy expenditure and body temperature regulation: induction of hypo- and hyperthermia (species dependent); 4—Locomotor activity: forepaw tremor in rats, “wet dog shaking activity”, muscle tone improvement (antagonism of induced relaxation); 5—Cardiovascular effects: increase blood pressure and heart rate; 6—Respiratory effects: increase respiratory rate; 7—Gastrointestinal effects: increase/decrease motility (species dependent), increase gastric acid secretion, increase gastric emptying; 8—Anorexic: decrease food and water intake [22].

2. Extrahypothalamic TRH and hypertension

In this section, we will discuss the current evidence indicating that extrahypothalamic TRH subserves a key role as a phasic neuroregulator of cardiovascular function and that is important as a central hypertensive neuropeptide not only in animal models of hypertension but also in essential and obesity-induced hypertension in rodents and man.

2.1. TRH in central cardiovascular regulation

The presence of TRH in brain nuclei involved in cardiovascular regulation [23], such as the preoptic area (POA), suggests that the tripeptide may play a role in modulating the cardiovascular function [24]. The POA that includes the anteroventral third ventricle region is crucial in regulating arterial blood pressure, dipsogenic behavior, antidiuretic hormone release, natremia and blood volume. Its destruction avoids the development of different forms of hypertension, such as that produced by DOCA-salt, sinoaortic denervation or lesion of the tractus solitarius nucleus [25]. In fact, microinjections of TRH intracere-

broventricularly (icv) or into the POA produce dose-dependent pressor effects [26,27].

It has been described that 3 ng of TRH caused a significant elevation of blood pressure and heart rate, whereas a higher 3-fold dose was needed to observe a significant increase in the respiration rate. The authors concluded that the tripeptide has profound physiological effects and that TRH, given centrally, is a potent hypertensive, chronotropic and tachypneic agent in the anaesthetized rat [28].

In addition, we demonstrated that TRH (0.5–4 μg) injected into the lateral septal region of the rat brain, did not elicit any significant change in the arterial blood pressure, but potentiated the effect of acetylcholine suggesting a neuromodulator role of TRH on cholinergic neurotransmission. This phenomenon is apparently due to an increase of the number of muscarinic receptors in the lateral septal area of the rat brain [29]. In fact, we observed a similar effect in the gastrointestinal tract [30].

Central cardiovascular effects of TRH seem to be mediated mainly by sympathetic activation. To study peripheral mechanisms underlying cardiovascular responses to thyrotropin-releasing hormone, Mattila et al. recorded the effects of icv infusions of TRH in urethane-anesthetized rats after various drug pretreatments, nephrectomy or renal denervation. TRH invariably increased blood pressure, heart rate and sympathetic nerve activity. After alpha-1 adrenergic blockade with prazosin, the pressor responses to TRH were delayed in onset and reduced in magnitude [31]. Therefore, because pressor responses to TRH were always accompanied by increased sympathetic nerve firing and were completely abolished after pentolinium-induced ganglioplegia, they were attributed solely to sympathetic hyperactivity [32]. The cardiovascular and endocrine activity of three analogs of thyrotropin releasing hormone, 4-nitro-imidazole TRH (4-nitro-TRH), 2-trifluoro-methyl-imidazole TRH (2-TFM-TRH) and 4-trifluoro-methyl-imidazole TRH (4-TFM-TRH), was compared to TRH in conscious rats. The results suggested that the receptors for TRH-elicited PRL release differ from TRH-receptors involved in its cardiovascular actions [27]. A solid body of evidence indicates that the effect of TRH on sympathetic activation is mediated by several classical neurotransmitter systems, such as muscarinic cholinergic, cathecolaminergic and serotoninergic systems, throughout the entire CNS including the brain, brain stem and spinal cord [33–35]. In addition, TRH is colocalized with other neurotransmitters and or neuromodulators including serotonin, P substance, dopamine and NPY [36,37].

2.2. Diencepahlic TRH in the spontaneously hypertensive rats (SHR)

In the spontaneous hypertension of rats, many neurochemical abnormalities have been described, in particular involving the cholinergic system [38]. Since the above-mentioned TRH–acetylcholine interaction in several central nuclei [29,39], we decided to explore the endogenous activity of the TRH system in one of the central areas of cardiovascular regulation, the POA [25]. Our study [40] showed for the first time that SHR have a 2-fold increase in TRH content of the POA with respect to its control normotensive strain, Wistar–Kyoto (WKY) rats. Two possibilities could explain these results: a) a reduced TRH release or b) an enhanced synthesis. Northern blot analysis indicated that the TRH precursor mRNA is more abundant in the POA of SHR than in age-matched WKY rats pointing out a probable increase in TRH synthesis. The difference was more apparent in adult rats that have developed hypertension but it was also seen in animals during the prehypertensive state. Since SHR also showed a significant increase of the TRH concentration in the cerebrospinal fluid, we postulated that the TRH release may also be elevated in this hypertensive condition although a reduced degradation cannot be ruled out. In any case, the elevated TRH concentration in SHR indicated an enhanced central overall TRH presynaptic hyperactivity. In addition, we found that the POA TRH receptor number is significantly increased in SHR with respect to normotensive rats indicating that there may also be an augmented postsynaptic TRH sensitivity. These results agreed with those of Bansinath et al. [41] and Bhargava et al. [42], who have reported a greater magnitude of TRH effects on arterial blood pressure and body temperature in SHR compared to WKY rats that can be related to an increase in the TRH receptor number in hypothalamus and striatum of 6 week-old SHR compared to age-matched WKY rats. Although our study was not focused on the TRH activity of the hypothalamic–pituitary axis, our data show an increased basal plasma TSH level and a greater TSH response to intraperitoneally administered TRH in SHR than in WKY rats but similar plasmatic T_3 and T_4 levels. Because until now, no specific antagonist is available for the TRH receptor in that previous piece of work, as a first approach, we have analyzed the effect of a polyclonal antibody against TRH injected peripherally or intracerebroventricularly. The hypotensive effect of this polyclonal semipurified anti-TRH IgG infused either peripherally or intracerebroventricularly in SHR argues in favor of a pathogenic role of TRH in this condition. The action of the antibody was modest and transient, probably due to the presence of neutralizing endogenous TRH and the difficulty of big molecules to reach the synaptic space, although the POA is considered to be outside of the blood brain barrier, and this fact may explain, at least in part, the hypotensive effect of the intravenously injected TRH antibody. Similar results were reported by Nurminen using a long term passive immunization with a heterologous antibody to TRH [43].

However, it remained controversial as to whether the increased expression of the extrahypothalamic TRH system in the spontaneously hypertensive strain is the cause of the

elevated arterial blood pressure. Therefore, we have reported [44] that intracerebroventricular antisense (AS) treatment with a phosphotioate 23-mers oligonucleotide targeted to bases 20–42 encompassing the translation initiation codon of the rat TRH precursor gene significantly diminished up to 72 h and in a dose-dependent manner the increased diencephalic TRH content while normalized systolic blood pressure (SABP) in the SHR compared to WKY rats. Although, basal TSH was higher in SHR compared to WKY rats and this difference disappeared after antisense treatment, no differences were observed in plasma T4 or T3 between strains with or without AS treatment indicating that the effect of the AS on SABP was independent of the thyroid status.

On the other hand, the vast interactions between neurotransmitters and neuropeptide systems involved in the cardiovascular regulation are still unknown. The brain renin–angiotensin system is considered one of the most important in blood pressure control either by a direct action or through the activation of other neurohumoral mechanisms [45]. The augmented central angiotensin (AII) production with an increased AII receptor number is a common reported feature of the SHR [46]. A growing body of evidence shows that AS treatments against angiotensinogen, angiotensin converting enzyme or angiotensin II receptor subtype 1 (AT1R) normalize blood pressure in the SHR [47–51]. Therefore, we measured diencephalic AII content in this model. We found that TRH AS treatment decreases the elevated diencephalic AII content to values comparable to the WKY control rats without any effects in the WKY control animals. Taking into account the theoretical high specificity of the TRH AS treatment (100% homology with the preproTRH gene), the decrease in the diencephalic AII content suggests that the TRH may exert some regulation over the angiotensin system. In this strain, a long lasting antihypertensive effects of an AS treatment against the TRH-R gene was also obtained by Suzuki et al. [52].

These results pointed out that TRH plays an important role in the development and/or maintenance of hypertension in SHR.

2.3. Diencephalic TRH over-expression induced hypertension

From our previous SHR study, the question arose whether an increased activity of the TRH system produces hypertension only in the abnormal biochemical environment that characterizes the central nervous system of SHR or if it is also able to induce high blood pressure in normal animals. Then, to investigate whether an increase in central TRH activity produces hypertension we studied the effect of the preproTRH over-production induced by icv transfection with a naked eukaryotic expression plasmid vector which encodes preproTRH (pCT). Northern blot analysis and reverse transcriptase-polymerase chain reaction showed that pCT was transcribed in vitro and in vivo. At 24, 48 and 72 h, pCT (100 μg) significantly and in a dose-dependent manner increased the diencephalic TRH content (37%, 84% and 49%), and SABP (42±3, 50±2 and 22±2 mmHg,), respectively, with respect to the vector without the preproTRH cDNA insert as measured by radioimmunoassay and the pletismographic method, in awake animals. In addition, using immunohistochemistry we found that the increase of TRH was produced in circunventricular areas where the tripeptide is normally expressed. To further analyze the specificity of these effects we studied the actions of 23-mers sense (S), AS, and 3′ self-stabilized sense (Ss) and antisense (ASs) phosphothioates oligonucleotides against the initiation codon region. Only ASs inhibited the increase of TRH content and SABP induced by pCT treatment. In addition, pCT-induced hypertension seems not to be mediated by central AII or serum TSH [53].

These experiments, demonstrated that central TRH overproduction in the periventricular area produces hypertension in normal rats that can be reversed by specific antisense treatment.

2.4. Extrahypothalamic TRH in human hypertension

Even though in essential hypertension, a polygenic and multifactorial syndrome, several genes interact with the environment to produce high blood pressure [54], our results prompted us to study the possible participation of the TRH system in human hypertension. The human TRH receptor (hTRH-R) belongs to the G protein-coupled seven transmembrane domain receptor superfamily. As seen in several neuroendocrine diseases, mutations of these receptors may result in constitutive activation. Since it has been demonstrated that hypertensive patients have a blunted TSH response to TRH injection suggesting a defect in the hTRH-R [55], we postulated that the hTRH-R gene is involved in essential hypertension. We studied two independent populations from different geographic regions of our country, a sample of adult subjects from a referral clinic and a population-based sample of high school students. In searching for molecular variants of the TRH-R, we disclosed that a polymorphic TG dinucleotide repeat (STR) at −68 bp and a novel single nucleotide polymorphism, a G→C conversion at −221 bp from the transcription initiation site, located in the promoter of the TRH-R are associated with essential hypertension. As STRs detected in gene promoters are potential Z-DNA forming sequences and seem to affect gene expression, we studied the potential different transcriptional activity of these TRH-R promoter variants and found that the S/-221C allele has a higher affinity than L/G-221 allele to nuclear protein factor(s).

Our findings support the hypothesis that the hTRH-R gene participates in the etiopathogenesis of essential hypertension [56].

3. TRH in obesity-induced hypertension

Obesity is a major risk factor for essential hypertension. Conversely, hypertensive patients tend to be more obese than normotensive subjects [57,58]. On the other hand, weight reduction is an effective way to lower arterial blood pressure (ABP) in obese hypertensive patients suggesting an important association between weight and ABP homeostasis [59].

3.1. Leptin–TRH interaction

A cumulative body of evidence have also suggested that obesity-induced hypertension may be due to, among other factors, an increased sympathetic outflow [60]. However the mechanisms of this association are poorly understood. In addition, leptin is an adipocyte-derived hormone that is involved in the regulation of food intake and body weight with the hypothalamus as a primary target of its action [61]. Leptin effects include increases in the overall sympathetic activity [62]. As reported by Ahima et al. leptin also counteracts the starvation-induced suppression of thyroid hormone apparently by up-regulating the expression of the preproTRH gene [63,64]. Then, we measured plasma leptin levels in male Wistar rats made hypertensive by periventricular TRH over-production induced by icv injection of the eukaryotic expression plasmid containing the preproTRH cDNA (pCT). We showed that pCT decreased leptin plasma levels and that a preproTRH AS treatment reverted this effect whereas an AS oligodeoxynucleotide with an inverted sequence used as control did not. Both male and female SHR displayed lower levels of circulating leptin than their sex- and age-matched WKY controls. These differences were abated by the preproTRH AS treatment. Conversely, in Wistar rats icv leptin induced a long-lasting pressor effect that was not observed in preproTRH AS-pretreated rats, but it was still present in inverted AS oligonucleotide a-treated animals.

These data indicated that leptin is decreased in TRH-induced hypertension that may over time lead to a compensatory gain in adipose tissue.

3.2. Diencephalic TRH in a rat model of obesity-induced hypertension

We then proposed that as leptin increases central TRH synthesis and release, obesity may rise ABP through TRH system activation, thus the TRH–leptin interaction may contribute to the strong association between hypertension and obesity [65]. To further explore this assumption, we developed a rat model with obesity-induced hypertension by a high fat diet [66]. Then, we showed that in rats made obese by a high fat diet, there was a correlation of the increased peritoneal adipose tissue and circulating leptin levels. Unsurprisingly, the higher levels of leptin were associated with an increase in SABP probably due to an increased sympathetic outflow since obese animals have an elevated concentration of plasma *O*-methyl metabolites of cathecolamines such as normetanephrine and metanephrine. These results are in agreement with the fact that acute and chronic leptin treatments can increase ABP in anesthetized and conscious rats and in *ob/ob* mice [62,65]. As hypothesized, we observed that in obese rats the increase in SABP was accompanied by an elevation in diencephalic TRH levels. It can be argued that this effect was directly due to the increase in leptin since Harris et al. reported that leptin up-regulates TRH gene expression acting on its promoter either through the activation of a cAMP response element or a Stat-response element [67]. As recently pointed out by other groups, leptin action on TRH gene expression can be mediated by increasing α-MSH or decreasing neuropeptide Y [68,69].

In fact, we found a significant correlation between diencephalic TRH levels and plasma leptin.

In order to explore whether the increase in SABP was related to the elevated diencephalic TRH content, we treated obese animals with an icv injection of a preproTRH AS. We observed that the AS injection normalized SABP 24 and 48 h post-treatment. Furthermore, at 48 h after AS treatment we confirmed that this effect on SABP was due to an action of the preproTRH AS on the TRH system by showing that the diencephalic TRH content was also diminished to levels similar to the levels found in control animals. As we previously reported, TRH AS had no effects on either diencephalic TRH content or SABP in control rats probably showing that TRH do not play a tonic role in controlling ABP under basal circumstances [40,44,53].

One possible site of action of the icv AS is the hypothalamus–pituitary axis, where alterations in the TRH synthesis might affect thyroid status indirectly influencing cardiovascular function. But this explanation seems unlikely since we found no change in thyroid hormone levels prior or after AS treatment. Furthermore, in our hand, AS treatment was effective and selective in decreasing the elevated concentrations of normetanephrine and metanephrine in obese animals that adds additional evidence to the existence of a TRH-dependent elevation of SABP mediated by sympathetic overactivity. As TRH is a potent prolactin releaser [70], it can be hypothesized that AS treatment may decrease SABP by affecting prolactin levels. We cannot reject that possibility but this seems improbable since prolactin does not alter SABP directly and may require a week to potentiate the pressor effect of norepinephrine [71].

In addition, following reports describing the long lasting effect of the small interfering RNA (siRNA) in diminishing target gene expression [72] we recently showed that icv siRNA against TRH precursor gene decreased the elevated diencephalic TRH content whereas normalized the higher SABP up to four weeks in rats with obesity-induced hypertension proving that siRNA is a potent tool to get a long lasting knocking down of candidate genes in mammals in vivo (500 times less siRNA than AS was necessary to

decrease in a similar magnitude SABP for a much longer period of time) (manuscript in preparation).

On the other hand, leptin gene expression and secretion are not only nutritionally but also hormonally regulated; they are increased by overfeeding, high fat diet, insulin, and glucocorticoids and decreased by fasting and catecholamines [73,74]. Therefore, it is tempting to speculate on whether there is a reciprocal interaction between periventricular TRH and leptin levels mediated by the sympathetic outflow. As published recently, our previous results taken together suggest that an increased periventricular TRH activity induced hypertension and decreased plasma leptin levels in two different experimental rat models of hypertension [65]. As spontaneous mutations in the leptin receptor gene in *db/db* mice and *fa/fa* rats producing defective leptin receptors lead to severe obesity, this could imply that diminished leptin levels in the TRH-induced hypertensive state may cause an increase in food intake and a decrease in leptin-mediated energy expenditure, hence producing a compensatory increase in adipose tissue. This may explain, at least in part, the tendency of hypertensive subjects to gain the so-called central adiposity.

4. Conclusion

We believe that TRH plays a central role in cardiovascular regulation under pathological conditions such as essential hypertension. In addition, as leptin produces central TRH synthesis and release [65,67], we propose that the obesity-related leptin elevation may induce hypertension through the TRH system activation which, in turn, increases symphatetic nerve activity. Recently, the concept has been raised that in some obese, leptin resistant models, there is a preservation of sympathoexcitatory actions of leptin despite resistance to the anorexigenic and metabolic action of leptin[75]. If this concept proves to be true, TRH may be the mediator of this preserved pathway activated by leptin. At any rate, although more experiments are necessary to delineate this complex TRH–leptin interaction, it may contribute, at least in part, to the strong association between hypertension and obesity. In addition, these studies open the intriguing possibility that an elevation of ABP is a putative side-effect of any treatment of obesity with fenfluramine-like drugs that may act by increasing the activity of the POMC–αMSH system in the arcuate nucleus of the hypothalamus [76]. Then the therapeutic management of obesity appears more challenging than ever.

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