Cardiac Delivery of Interference RNA for Thyrotropin-Releasing Hormone Inhibits Hypertrophy in Spontaneously Hypertensive Rat

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See related article, pp 103–109

Thyrotropin-releasing hormone (TRH) is a tripeptide synthesized in the paraventricular nucleus of the hypothalamus and is best known for its classic role in stimulating the release of thyroid-stimulating hormone (TSH) and prolactin from the anterior pituitary gland. By stimulating the release of TSH, TRH regulates thyroxine (T4) and triiodothyronine (T3) production and secretion into the bloodstream. The presence of a TRH system in the paraventricular and preoptic nuclei of the hypothalamus suggests a direct role of TRH in cardiovascular physiology. Indeed, blood pressure (BP), heart rate, and contractility increase after intracerebroventricular1 or intravenous2 administration of TRH in rats. Although administration of exogenous TRH at pharmacological doses has helped to identify the potential roles of TRH, further elucidation of endogenous TRH function and mediation by its 2 receptors in rodents (in humans, only 1 TRH receptor has been identified thus far) have been hampered because of the lack of receptor subtype-selective antagonists. Furthermore, these observations are consistent with the fact that the heart is a major target organ for thyroid hormones, and that there are marked changes in cardiac function and structure in patients with hypothyroidism or hyperthyroidism.3

Spontaneously hypertensive rats (SHR) are used as a model of human essential hypertension. It presents an increase in both TRH content and TRH precursor mRNA abundance in the preoptic area, with a higher cerebrospinal fluid TRH concentration and TRH receptor number in this area.4 This model demonstrates a gradual progression of myocardial hypertrophy, fibrosis, left ventricular (LV) dysfunction, and heart failure. Persistent hypertension begins when rats reach ~2 months of age and is followed by a relatively long period of stable, compensated hypertrophy. By 18 to 24 months, male SHR develop heart failure and exhibit a marked upregulation of genes that encode for extracellular matrix components. Also associated with this alteration in gene expression is an increase in fibrosis and impaired LV function. The complete TRH system is present in the heart and includes pro-TRH transcript and peptide5 and TRH receptor.6 The presence of positive inotropic effects has helped elucidate the functionalities of TRH and TRH receptor in an isolated rat heart preparation and in isolated adult rat ventricular myocytes.4 Because elevation of the TRH system in the rat heart has been reported previously in experimental myocardial infarction and linked with possible cardiac damage,2 Schuman et al7 proposed in this issue of Hypertension that inhibition of local TRH synthesis will prevent LV hypertrophy in SHR. During the shift from normal (7 weeks of age) to the hypertrophic (18 weeks of age) phenotype, they initially observed a 15-fold increase in TRH mRNA expression in the left ventricle but not in other chambers of the heart. Consequently, they predicted that blocking this TRH elevation in the left ventricle would have an effect on the progression of hypertrophy. The methodology used for this purpose is a logical continuation of previous work on the effects of inhibition of TRH synthesis in the brain.4 The authors already reported that reducing TRH production in the diencephalic region by administration of antisense phosphorothioate oligonucleotides against the translation initiation codon region of the TRH-precursor gene normalizes the systolic BP in SHR, whereas sense or vehicle treatment showed no effect.4 In the current study,7 the in vivo intracardiac treatment with naked interference RNA (iRNA) had no effect on BP in SHR; it, however, did normalize the heart/body weight ratio, prevented LV fibrosis and cardiomyocyte enlargement, and attenuated the expression of B-type natriuretic peptide, a recognized marker of hypertrophy.

The present study demonstrates successful prevention of cardiac hypertrophy in a rat model by cardiac-targeted iRNA ablation of TRH. This interesting observation raises a number of questions including one raised by the authors7: Is inhibition of LV hypertrophy in SHR by specific iRNA treatment beneficial or does it create an increased risk of developing more severe heart failure, in conditions of elevated BP? According to Laplace’s law, an increased wall thickness of the LV chamber reduces wall stress; the development of cardiac hypertrophy can be considered an adaptive response to a variety of stimuli, most commonly altered workload. Therefore, impairment of this compensatory mechanism can lead to LV dysfunction. For example, inhibition of cardiac hypertrophy in a mouse model of pressure overload induced by administration of cyclosporine A, an inhibitor of Ca2+-regulated phosphatases (calcineurin), was associated with increased heart failure.8 The observation that iRNA administration effectively prevents myocardial fibrosis and cardiac...
hypertrophy even in the absence of a fall in BP implies tissue-specific autocrine/paracrine mechanisms influenced by the treatment. Therefore, hemodynamic changes alone could not account for the effect on cardiac hypertrophy.

Is the upregulation of TRH expression and activity an important step in predisposing the left ventricle to hypertrophy in SHR? The 2 types of rodent TRH receptors exhibit similar affinities for TRH and appear to signal via similar transduction pathways, primarily mediated by Gq/G11 and phospholipase C. The Gq/G11 family of G proteins is centrally involved in myocardial hypertrophy. G protein–coupled receptors such as the α1-adrenergic receptor, the angiotensin AT1 receptor, or the endothelin ET_A receptor, act through Gq/G11 to induce cardiomyocyte hypertrophy and fibroblast proliferation. They are coupled to several downstream effectors including the calcineurin/NFAT (nuclear factor of activated T lymphocytes) pathway, protein kinase C (PKC) isoforms, and mitogen-activated protein (MAP) kinase pathways. Data from previous work indicate that fibroblasts are the primary source of pro-TRH signal in the heart and that expression of pro-TRH in cultured adult rat cardiac fibroblasts is stimulated by a variety of adrenergic agonists. Because TRH receptors are also present on cardiac fibroblasts and since TRH inhibition prevents accumulation of these cells in the heart, we can speculate that TRH stimulates the growth of these cells via autocrine/paracrine mechanisms. Because TRH regulates cardiac myocyte function, TRH release from fibroblasts may contribute to the cross-talk between cardiac fibroblasts and myocytes during the development of LV hypertrophy in SHR (Figure A). Although cardiomyocyte hypertrophy may result from multiple causes, defective Ca^{2+} homeostasis has been identified as an important final common pathway. In fact, multiple kinase pathways that stimulate cell proliferation, including PKC, Ca^{2+}/calmodulin-dependent kinase, and MAP kinase, are activated by TRH. TRH receptor activation of Ca^{2+}\textsuperscript{2+} in cardiomyocytes and fibroblasts may possibly change cellular Ca^{2+} homeostasis and contribute to the LV hypertrophy (Figure B).

The results presented by Schuman et al demonstrate that local TRH synthesis is a major stimulus for collagen production by cardiac fibroblasts in the myocardium of SHR, rather than the LV workload against an increased afterload. Since this function is usually attributed to angiotensin 2, further clarification is needed concerning the interaction of TRH with the renin-angiotensin system. The renin-angiotensin system in the development of LV hypertrophy, as reported by Bacova et al, indicated that TRH receptors enhance fibroblast proliferation and secretion. TRH activation of myocytes provokes cell hypertrophy and release of B-type natriuretic peptide (BNP). B, Model showing potential hypertrophy/proliferation transcription pathways. AP-1 indicates activator protein 1; CaM, calmodulin; DAG, diacylglycerol; IP3, inositol-1,4,5-triphosphate; MAPK, MAP kinase; MEF, myocyte-enhancing factor; NFAT, nuclear factor of activated T lymphocytes; PLC, phospholipase C; SRF, serum response factor.

The TRH gene in humans and rodents encodes for multiple copies of TRH. After its transcription and translation, the 26-kDa TRH prohormone is sequentially modified by prohormone convertases, thereby giving rise to a variety of cleavage products of smaller molecular weight. These proteins are further modified by enzymes. Some of the precursors of TRH have biological activity, and their inhibition by iRNA treatment could also potentially alleviate the hypertrophic processes in SHR.

**Perspectives**

This finding has both specific and general implications. Generally, it adds another function to the already imposing list of properties of TRH. Because there are no known antagonists of TRH, the iRNA approach highlighted in this study is likely to be the useful technique available to investigate the roles of TRH, and as such, it can be added to the limited repertoire of techniques available to study TRH. As the authors mentioned, this in vivo intracardiac treatment, which involves repeated injections of naked iRNA, is not
clinically useful. However, new evidence indicates that iRNA can be introduced to the heart via viral vector where successful treatment of heart failure was demonstrated. Simple intravenous injection of phospholamban iRNA carried by adeno-associated virus restored cardiac function and reduced dilatation, cardiomyocyte hypertrophy, and fibrosis in a rat model of aortic banding by iRNA targeting of phospholamban.11 In summary, Schuman et al11 raise the hopes that novel strategies may be designed to target LV hypertrophy.

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None.

References
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