Thyrotropin-Releasing Hormone Is Induced in the Left Ventricle of Rats With Heart Failure and Can Provide Inotropic Support to the Failing Heart

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Background—We reported previously that left ventricular gene expression for thyrotropin-releasing hormone (TRH) precursor was increased in rats with heart failure 8 weeks after myocardial infarction (MI) and that early ACE inhibition tended to cause further myocardial induction of this gene.

Methods and Results—Here, we show that after MI, the expression of pro-TRH is induced in the heart coordinately with the protease PC1, an important enzyme in TRH biosynthesis. Pro-TRH gene expression was induced in cardiac interstitial cells after MI, and this effect was restricted to the heart, because no increase in TRH mRNA abundance was observed in the hypothalamus, kidney, or lung. Transcript abundance of pro-TRH can be increased in cultured cardiac fibroblasts by several adrenergic agonists, indicating that the adrenergic axis may play a regulatory role in cardiac TRH production. Acute intravenous administration of TRH to rats with ischemic cardiomyopathy caused a significant increase in heart rate, mean arterial pressure, cardiac output, stroke volume, and cardiac contractility.

Conclusions—Taken together, these results indicate that TRH is specifically induced in the heart after MI and that it can increase cardiac performance in rats with ischemic cardiomyopathy. Thus, in addition to catecholamine and angiotensin II, pro-TRH/TRH may be another important axis that affects hemodynamics and cardiac function in heart failure. (Circulation. 2004;109:2240-2245.)

Key Words: hormones ■ heart failure ■ myocardial infarction ■ inotropic agents

Thyrotropin-releasing hormone (TRH; pyro-Glu-His-ProNH₂) is best known for its role in the thyroid hormone axis. It is produced in the hypothalamus and regulates the production of thyrotropin in the pituitary. Thyrotropin in turn stimulates the production of the thyroid hormones T₄ and T₃ from thyroglobulin in the thyroid gland. TRH is also produced in peripheral organs, however, and has been shown to have central and peripheral biological effects that are mediated by TRH receptors and independent of thyroid hormone production (see Nillni and Sevarino¹ for a review of this literature).

TRH has been shown to have effects on the cardiovascular system in rodents. Blood pressure and heart rate were increased in anesthetized²,³ and conscious⁴ rats by intracerebroventricular (ICV) administration of TRH, and in anesthetized rats, ICV TRH increased blood flow to most organs.⁵ The preponderance of data indicate that central TRH regulation of cardiovascular function is mediated through the sympathetic nerves and the adrenal medulla.⁶

TRH and the TRH receptor are also expressed in the heart. Carnell et al⁶ showed that mRNA for pro-TRH and TRH peptide were produced in the heart, and Wilber and Xu⁷ reported finding mRNA for the TRH receptor in the heart as well as specific and saturable binding of H-3CH₃His-TRH to heart membrane preparations. Furthermore, TRH was shown to have direct inotropic effects in an isolated rat heart preparation⁸ and on isolated adult rat ventricular myocytes.⁷

Using DNA microarrays, we previously identified pro-TRH as 1 of 31 genes induced in the left ventricle (LV) of rats with heart failure, 8 weeks after myocardial infarction (MI).⁹ Interestingly, we found that early ACE inhibition, which normalized cardiac mass and function in this model, tended to further increase the LV expression of pro-TRH. This was in contrast to the general trend of the ACE inhibitor to normalize the changes in gene expression induced by MI. Here, we show that the cardiac contractility and output of the failing rat heart can be increased by TRH, and we propose that LV production of TRH can help to provide inotropic support during ischemic cardiomyopathy and compensate for the lack of angiotensin II during therapeutic ACE inhibition.
Methods

All animal-use protocols conformed to the guiding principles of the American Physiology Society and were approved by Genentech’s Institutional Animal Care and Use Committee.

Animal Model

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc; Wilmington, Mass; 8 weeks of age) were anesthetized with ketamine hydrochloride (100 mg/kg IP) and xylazine (10 mg/kg IP), intubated via tracheostomy, and ventilated with a respirator (Harvard Apparatus, model 683). MI was produced by coronary artery ligation as described previously. Briefly, after a left-sided thoracotomy, the left coronary artery was ligated ~2 mm from its origin with a 7-0 silk suture. In experiments in which reperfusion was allowed, the suture was released 2 hours after ligation. For time points up to 1 day after surgery, rats with evident infarctions were identified by measuring troponin-T released into the plasma. For time points ≥1 day, animals with ECG evidence of infarction were included in the study.

Assessment of Cardiac Performance

Mean arterial pressure (MAP) and heart rate (HR) were measured by catheterization. After the rats had been anesthetized as described above, catheters (PE-10 fused with PE-50) were implanted into the abdominal aorta via the right femoral artery for measurement of MAP and HR and into the right femoral vein for TRH administration. Cardiac output was measured by an ultrasonic probe as previously described. The ultrasonic perivascular flow probe (25165, Transonic Systems Inc) was placed around the ascending aorta through a right-sided thoracotomy. The flow probe cable was exteriorized at the back of the neck, and the cable connector was sutured and fixed in place. The chest was closed and the tracheal incision sutured after extubation.

One day after surgery, MAP and HR were measured and cardiac output was determined with a model T 206 flowmeter (Transonic Systems Inc) while the animals were conscious and unrestrained. Stroke volume was calculated as cardiac output divided by HR and systemic vascular resistance as MAP divided by cardiac output. After hemodynamic stabilization, rats received intravenous injection of TRH (3.3 mg/kg) or saline vehicle. The dose was chosen on the basis of preliminary studies of the dose response of TRH on MAP and HR in conscious rats. The vehicle group included 3 MI and 3 sham-operated rats. The hemodynamic parameters were simultaneously and continuously recorded before and after the injection.

To avoid a possible influence of the LV catheter on cardiac output, LV dP/dt was measured in a separate group of MI and sham rats. A microtip catheter transducer (SPR-629, 1.8 F, Millar) was implanted into the LV through the right carotid artery. One day after implantation, LV dP/dt was measured before and after intravenous injection of TRH into conscious animals.

Results are expressed as mean ± SEM. One-way ANOVA was performed to assess differences in parameters at the same time point between groups and to compare changes over time within each group. A value of P<0.05 was considered to be statistically significant.

At the end of the experiments, the LV was dissected and saved for histological evaluation of infarct size in 6 MI rats selected at random. The infarct size was determined with a planimeter Digital Image Analyzer as described previously. The infarct size was 33 ± 2% of the LV.

Isolation of Cardiac Myocytes and Fibroblasts

Myocyte Collection

Ventricular myocytes were harvested from adult rats according to the protocol described by Lai et al, with slight modifications. Briefly, 2 adult rats weighing ~280 g were anesthetized with Nembutal (pentobarbital sodium). Their hearts were removed and affixed on the Langendorff apparatus and were perfused at 37°C with Krebs buffer (in mmol/L: 110 NaCl, 2.6 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄ · 7H₂O, 25 NaHCO₃, and 11 glucose). A solution of 60 mg collagenase/12.5 µL of 100 mmol/L CaCl₂ in ~50 mL Krebs buffer was then recirculated through the hearts for 25 minutes. The hearts were removed from the apparatus, and the atria and connective tissue were dissected out. The ventricles of both hearts were cut into small pieces and placed in a solution containing 800 mg of BSA dissolved in 30 mL of the circulation buffer from the previous step. The mixture was incubated in a 37°C shaking water bath for 5 minutes, and then digested tissue was sieved through a 200-µm nylon mesh to collect free cells. Remaining tissue was digested for an additional 5 minutes in a fresh collagenase/BSA solution, and cells were pooled together with the first digestion. The cells were spun for 3 minutes at 600 rpm. The supernatant was aspirated, and the pellet was briefly washed with 10 mL of 0.3 mg/mL soybean trypsin inhibitor diluted in Krebs buffer supplemented with 0.2 mmol/L CaCl₂. The cells were spun for 3 minutes at 600 rpm. The pellet was resuspended in 20 mL of Krebs buffer containing 0.5 mmol/L CaCl₂. Cells were then passed through a Krebs buffer/BSA gradient for 15 minutes, and myocytes were resuspended in glucose-free medium 199 with NaHCO₃, and phenol red and were pooled. To calculate the amount of the lysis buffer to be used in the next step, cell number was estimated by use of a hemacytometer slide. Cells were spun, and the pellet was resuspended in 2.4 mL of the Qiangen lysis buffer RLT (Qiagen Mini Kit, Catalog No. 74124). The lysate was split into 4 samples of 600 µL each. The samples were then passed through Qiangen Qashredder columns (Catalog No. 79654) before the animal cell protocol recommended by Qiangen was continued.

Fibroblast Collection and Treatment

Fibroblast harvest started with the incubation of 2 perfused hearts in a 37°C shaking water bath in 60 mL of the digestion mixture (1.6 g BSA, 0.06 g collagenase, 75 µL 0.1 mol/L CaCl₂ in Krebs buffer). The tissue was triturated with a 10-mL pipette every hour. After 3 hours, the mixture was spun at 600 rpm for 3 minutes. The supernatant containing small cells was transferred to a new tube and spun again at 1000 rpm for 10 minutes. The pellet was resuspended in 20 mL of fibroblast media (6.69 g DMEM, 5.31 g F12, 1.2 g NaHCO₃, 0.4 g l-glutamine, 10 mL of 1 mol/L HEPES buffer, made up to a total volume of 1 L with water) containing 5% FBS and supplemented with 1% (vol/vol) penicillin-streptomycin (Gibco, Catalog No. 15140-122) and 1% insulin-transferrin-selenium-X (Gibco, Catalog No. 51500-056). Cells were plated in 2 culture flasks for 45 minutes to allow fibroblasts to attach to the bottom of the flask. Unattached cells were discarded by washing flasks twice with 15 mL of fresh media. Finally, fibroblasts were incubated in 15 mL of the 5% FBS-containing medium for 3 days, until cells became confluent and then were stripped off the plates with trypsin-EDTA (Gibco, Catalog No. 15400-054), spun, and washed with medium. At this stage, some cells were collected for RNA preparation, and cells from other harvests were replated at a density of 10⁴ cells/mL in the same medium and cultured for 24 hours before they were serum-starved for 1 day and then treated with the test substances for the next 24 hours. Experimental samples were harvested 24 hours after exposure to the treatment substances (day 1). At the same time, controls (no treatment) were also collected. Cultured fibroblasts were treated with the following factors: isoproterenol (10 µmol/L), dobutamine (10 µmol/L), epinephrine (10 µmol/L), norepinephrine (100 µmol/L), and phenylephrine (100 µmol/L). Control and treated cells (n=3) were lysed with 350 µL of RLT buffer and passed through Qashredder columns, and RNA was isolated by use of a Qiangen Mini Kit.

Gene Expression Analyses

RNA Preparation

Tissue samples were collected, frozen in liquid nitrogen, and stored at −70°C until used. For cardiac samples, the LV free wall (including the scar in rats with MI) and septum were collected. For experiments in which the regional expression was determined, the infarct scar, peri-infarct zone, and normal, uninvolved myocardium were dissected from the LV sample and treated separately. Similarly
located regions of control hearts were also collected. Total RNA was isolated with the RNeasy Maxi Kit (Qiagen, Catalog No. 75162).

**Real-Time RT-PCR**

Real-time reverse transcription–polymerase chain reaction (RT-PCR) was performed as described previously13 with the TaqMan model 7700 Sequence Detector (ABI). Expression values for each gene were normalized to either ribosomal protein L19 (RPL19) or total RNA as indicated. Results are expressed as mean ± SEM. One-way ANOVA was performed to assess differences in parameters at the same time point between groups and to compare changes over time within each group. An unpaired Student’s *t* test was used for comparison between 2 groups.

**In Situ Hybridization**

Sections 5 μm thick were deparaffinized, deproteinated in 4 μg/mL proteinase K for 30 minutes at 37°C, and processed further for in situ hybridization as previously described.14,15 Briefly, [33P]UTP-labeled sense and antisense riboprobes were hybridized at 55°C overnight, followed by a high-stringency wash at 55°C in 0.1 × standard saline citrate for 2 hours. Before being dipped into photographic emulsion, the dry glass slides were exposed for 3 days at room temperature to Kodak BioMax MR autoradiographic film. The slides were dipped in NTB2 nuclear track emulsion (Eastman Kodak), exposed in sealed plastic slide boxes containing desiccant for 4 weeks at 4°C, developed, and counterstained with hematoxylin and eosin.

**Results**

Real-time RT-PCR was used to study the time course of LV expression of pro-TRH mRNA in a rat model of cardiac ischemia (2 hours) followed by reperfusion (Figure 1).

Myocardial pro-TRH transcript levels were found to increase in a biphasic manner after MI. The first peak in pro-TRH expression occurred at 6 hours (≈4-fold induction). By 24 hours, pro-TRH expression had returned to control levels, and then it gradually increased, peaking at 30 days (≈10-fold induction).

TRH is produced from a 29-kDa precursor, pro-TRH, which contains 5 copies of the TRH progenitor sequence Gln-His-Pro-Gly. Biosynthesis of TRH is dependent on posttranslational processing of pro-TRH by convertases, mainly PC1 or PC2.16–19 We found that mRNA for pro-TRH and the TRH processing enzyme PC1 were induced coordinately in the LVs of rats after MI (Figure 2). At 8 weeks after MI, the greatest induction is in the infarct scar, but enhanced expression is also detectable in the peri-infarct zone and the normal, uninvolved myocardium.

Primary cultures of adult rat ventricular myocytes and fibroblasts were prepared from normal hearts and analyzed for pro-TRH mRNA. The data indicate that the fibroblasts are the primary source of the pro-TRH signal (Figure 3). These results were consistent with data obtained by in situ hybridization analysis of the MI heart that showed that interstitial cells, located predominantly in the infarct scar and infarct border zone, contained the pro-TRH mRNA (Figure 4).

Induction of TRH in rats after MI is cardiac specific. We examined pro-TRH transcript levels in the hypothalamus and thyrotropin mRNA levels in the pituitary of rats 8 weeks after MI and found no evidence of induction (data not shown). There was also no evidence of increased pro-TRH mRNA in the lungs or kidneys of the animals (data not shown).
The expression of pro-TRH in cultured adult rat cardiac fibroblasts can be regulated by a variety of adrenergic agonists. Pro-TRH mRNA abundance in isolated cardiac fibroblasts was increased \(\sim 5\) to 8-fold by isoproterenol, dobutamine, epinephrine, norepinephrine, and phenylephrine (Figure 5).

To determine the effects of TRH on hemodynamics and cardiac function in the setting of heart failure, TRH was administered acutely to rats with ischemic cardiomyopathy 30 days after left coronary artery ligation and to sham-operated control animals. Compared with the sham-operated group, rats with MI had heart failure, as shown by lower basal levels of cardiac output (sham, \(95.4 \pm 4.5\) versus MI, \(79.8 \pm 3.0\) mL/min, \(P<0.05\)), stroke volume (sham, \(0.2571 \pm 0.0096\) versus MI, \(0.2186 \pm 0.0128\) mL/beat, \(P<0.05\)), and LV contractility, as assessed by maximal dP/dt (sham, \(6670 \pm 742\) versus MI, \(4708 \pm 493\) mm Hg/s, \(P<0.05\)). MAP (sham, \(109 \pm 3.7\) versus MI, \(104.2 \pm 3.9\) mm Hg), HR (sham, \(371 \pm 10.2\) versus MI, \(370 \pm 20.9\) bpm), and systemic vascular resistance (sham, \(1.151 \pm 0.059\) versus MI, \(1.322 \pm 0.102\) mm Hg \(\cdot\) mL\(^{-1}\) \(\cdot\) min\(^{-1}\)) were not different between the 2 groups. Intravenous injection of TRH caused a rapid increase in MAP, HR, cardiac output, stroke volume, and LV dP/dt that peaked at 1 to 3 minutes and persisted for 10 to 20 minutes in both MI and sham rats (Figure 6). The increase in these parameters was similar in the 2 groups. In addition, intravenous TRH caused a transient decrease in systemic vascular resistance in both MI and sham control rats, because the TRH-induced increase in cardiac output exceeded the change in arterial pressure.
Discussion

Substantial amounts of cardiac muscle can be replaced with fibrous scar tissue after a heart attack, and there is a concomitant drop in cardiac performance. The resulting deficit in peripheral tissue perfusion stimulates catecholamine release and systemic and cardiac production of angiotensin II. These factors increase HR, MAP, and cardiac contractility. Although these physiological effects may initially improve cardiac performance, it is now clear that they have a long-term detrimental effect. Large-scale clinical trial results have proved that ACE inhibition and β-adrenergic receptor blockade have beneficial effects on the survival of heart failure patients.

In the present report, we describe another important axis that can affect cardiac performance during heart failure. We show that after MI, there is a sustained induction in the expression of LV pro-TRH mRNA and coordinate expression of an important enzyme in TRH biosynthesis, PC1. Although expression of TRH and PC1 is increased in all segments of the failing heart (scar and uninvolved myocardium), the greatest induction is in the infarct scar, and the source of the TRH appears to be fibroblasts. The induction of pro-TRH after MI is restricted to the heart and does not involve central upregulation of pro-TRH in the hypothalamus or induction of thyrotropin in the pituitary. We also show that adrenergic agonists can induce cardiac pro-TRH expression and that TRH can have a substantial effect on hemodynamics and cardiac function in the setting of heart failure. TRH can increase HR, MAP, and cardiac contractility in rats with ischemia-induced heart failure.

The implications of these findings for the pathophysiology and treatment of heart failure are intriguing. By analogy to the effects of angiotensin II and adrenergic agonists, it may be that long-term production of TRH in the failing heart is detrimental. If this is indeed the case, then inhibiting the effects of TRH may be a useful therapeutic approach. Because we observed a trend toward further induction of TRH precursor in rats treated with captopril, this approach may be a good complement to early ACE inhibition. However, targeting the TRH axis for therapeutic intervention may be problematic, because the indiscriminate inhibition of TRH would have adverse effects on thyroid hormone production and interfere with other important biological effects of TRH. Additional research will be necessary to determine whether there is anything unique to the cardiac TRH axis, such as specific processing of pro-TRH or cardiac-specific TRH receptor subtypes, that would enable a therapy targeted primarily to the heart.

References


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