Positive Inotropic Effect of Thyrotropin-Releasing Hormone on Isolated Rat Hearts

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Abstract. The effects of thyrotropin releasing hormone (TRH) on the contractility of electrically stimulated and perfused isolated rat hearts were investigated. TRH in the range of 0.1–10 μmol/l was found to exert a positive inotropic effect on cardiac contractility, which however qualitatively differed at lower vs. higher concentrations of the hormone: at 1 μmol/l, TRH was found to significantly enhance the rate of contraction as well as that of relaxation (by 23.2 ± 3.7 and $27.8 \pm 7.7\%$, respectively), which culminated in an increased peak contractile force. However, at 10 μmol/l, the positive inotropic effect of TRH (i.e. the increase in peak contractile force) was smaller than at 1 μmol/l, which apparently was due to both a reduced TRH-induced elevation in the rate of contraction ($12.4 \pm 3.2\%$) and a TRH-induced decrease in relaxation rate ($11.1 \pm 8.1\%$). Since TRH is expressed in the heart, the above findings suggest that, in addition to its CNS-mediated cardiovascular effects, TRH modulates cardiac contractility as an autocrine regulator in a concentration-dependent manner, which likely involves more than one TRH receptor and associated signaling pathway.

Key words: Thyrotropin releasing hormone — Rat heart — Cardiac contractility — Positive inotropic effect — Autocrine regulation

Introduction

Thyrotropin-releasing hormone (TRH) is a tripeptide that is best known for its involvement in the endocrine regulation of the pituitary-thyroid axis and its secretion by the hypothalamus. On the other hand, it is now well-documented that TRH is also present throughout the extrahypothalamic nervous system as well as in a number of peripheral tissues (Morley 1979). Accordingly, TRH has been shown

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to have non-pituitary-related functions, including the regulation of the cardiovascular system. The occurrence of TRH immunoreactivity (Eskay et al. 1983) and binding sites (Manaker et al. 1985) have been demonstrated in brain areas that are involved in the regulation of the cardiovasculature, and it has been shown that its intracerebroventricular injection improves cardiovascular function in both endotoxic and hemorrhagic shock (Holaday et al. 1981; Feuerstein et al. 1983; Holaday and Faden 1983), as well as it alters arterial pressure and heart rate (Beale et al. 1977; Delbarre et al. 1977; Koivusalo et al. 1979; Holaday et al. 1983).

The heart has been recently identified as a new locus for TRH expression (Carnell et al. 1992; Lathrop et al. 1994). Furthermore, it has been also found that TRH expression is regulated by different steroid hormones such as glucocorticoids and testosterone (Lathrop et al. 1994). Thus, by using an isolated rat heart model, we asked the question whether TRH, as a potential autocrine regulator of the heart, manifests any direct influence on cardiac contractility.

Materials and Methods

Surgical procedures

Hearts were isolated from Sprague-Dawley rats (260 g on average; Harlan Laboratories, Indianapolis, IN). The animals were killed by cervical dislocation, in accordance with the "Guiding Principles in the Care and Use of Animals". After midsternal thoracotomy, the hearts were excised and perfusion was initiated within 30 seconds with the means of a polyethylene catheter inserted into the aorta. Platinum electrodes were attached to the ventricles to control the contractile rate of the heart by electrical stimulation (Grass S8800) at an optimal frequency (240–300 BPM) which stabilized contractility in time.

Perfusion procedures

Each heart was perfused at a constant pressure of 60 cm H_2O, at which the flow rate through the heart was 3.5 ml/min, resulting in a stable contractility of control hearts for 5–6 hours. The perfusion fluid (modified Krebs-Henseleit solution) contained (in mmol/l) NaCl, 118; $NaHCO_3$, 25; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.1; and dextrose, 11.0 and was continuously aerated with a mixture of 95% O_2/5% CO_2 to maintain the pH at 7.35 (37°C). A stock solution of TRH (Sigma) dissolved in the perfusion medium was delivered with a syringe pump into the perfusion fluid close to where it entered the heart. The desired concentrations of TRH (as indicated in the text) in the perfusate that reached the heart were adjusted by controlling the flow rate of the pump according to the perfusion rate set separately.

Contractile force measurements

The apex of the heart was attached, by means of a ligature, to a (Statham UC-3) force transducer that was set up to measure isometric contractile force (F) and was interfaced to a chart recorder and an A/D device in a computer. Besides continuously monitoring the contractile status of the heart with the use of the chart recorder, digitized data was also collected in segments at a sampling rate of 1000 Hz, and analyzed with DaDisp Software. Each collected data segment was analyzed for maximal force, the rate of force development ($+dF/dt$) and the rate of relaxation ($-dF/dt$). Due to time-dependent changes in contractile force, the data presented as percentages of control values were compared to those measured prior to the addition of TRH, and are given as means ± S.E. The number of independent experiments (i.e. the number of rat hearts) was at least 4. Statistical significance (at $P < 0.05$) was evaluated using analysis of variance followed by Duncan's multiple range test. Before every experiment, the hearts were allowed to equilibrate for 30 min., during which time the resting force was set optimally to minimize the baseline drift.

Results

Fig. 1 shows 3-beat long representative segments of force-records before (Trace A) and 20 mins after (Trace B) the injection of 1 μmol/l TRH into the perfusion medium. Shortly after the administration of the hormone (see also Fig. 2), the peak force generated by the heart started increasing, and after 15–20 min reached a maximum which, on average, was 20–30% higher than the force generated by control hearts. This positive inotropic effect observed in the presence of 1 μmol/l

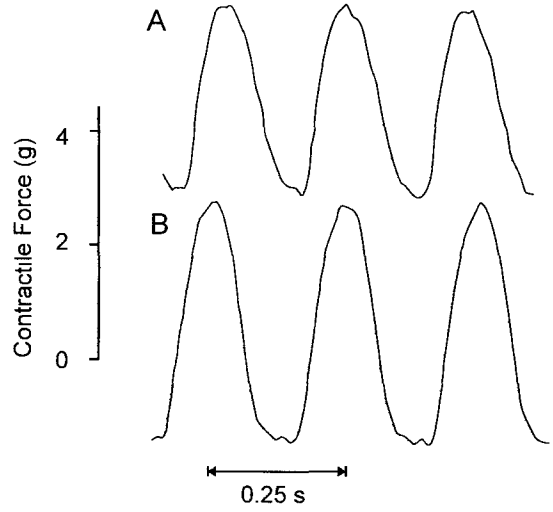

Figure 1. Positive inotropic effect of TRH on isolated rat hearts. After a 30 min. equilibration period, the heart stimulated at 240 BPM was perfused with 1 μmol/l TRH, and the force was monitored in segments. A: Record before (0 min), B: Record 20 min. after TRH was introduced. The abscissa applies to both traces.

TRH was apparently not associated with any change in the regularity in beating of the electrically paced heart (as it was clearly seen from the analysis of long stretches of time-resolved data-segments similar to those shown in Fig 1)

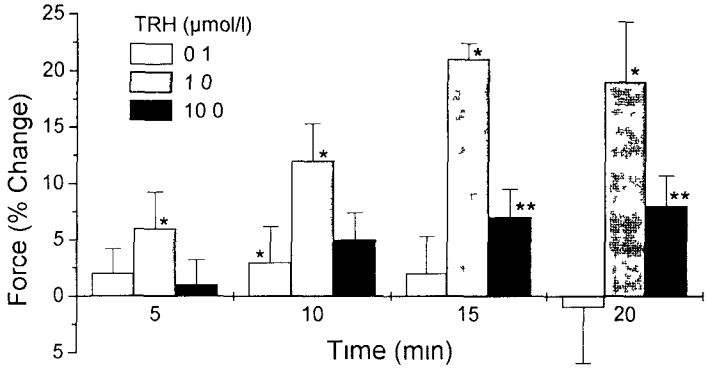

Figure 2. Force development in isolated rat hearts at various time points after being perfused with different concentrations of TRH The data of peak force from experiments similar to that shown in Fig 1 is plotted as the means ± S E (averaged from at least 4 independent measurements) The asterisks indicate statistical significance at $P < 0.05$ as compared to the control values Where indicated by two asterisks the difference was also significant (at $P < 0.05$) when compared to data points obtained with 1 μmol/l TRH

Fig 2 illustrates the temporal changes in the maximum (peak) force of isolated hearts in the presence of various concentrations of TRH As seen from the results in the Figure three properties of the TRH-induced changes on the contractility of isolated hearts were noticeable i) the maximal effect of TRH developed rather slowly as it required several minutes to become manifest and was more pronounced at higher TRH concentrations ii) the maximum force measured in the presence of 10 μmol/l TRH although still larger than that of the control was significantly reduced as compared to the force measured in the presence of 1 μmol/l TRH and iii) (as best seen from the measurements with 0 1 μmol/l TRH) the increase in maximal force was transient despite the continuous perfusion of the hearts with the hormone the positive motropic effect after reaching a maximum seemed to decrease over time Both the transient behavior of the TRH effect and its bell shaped concentration dependence suggest that TRH exerted a dual effect on contractility which probably involves the binding of the hormone to two sets of binding sites the saturation of the relatively high-affinity sites (i e at TRH concentrations up to 1 μmol/l) is likely responsible for the increase in peak contractile force while that of the lower-affinity site (at 10 μmol/l TRH) tends to result in the opposite effect (i e attenuation of the positive motropic influence)

A dual effect of TRH was also observed, when the influences of the hormone on the rate of contraction and relaxation were analyzed separately. As shown in Fig. 3, at 1 μmol/l TRH, the rates of contraction (dF/dt) and relaxation ($-dF/dt$) significantly increased as compared to the control rates, while at a higher concentration (10 μmol/l), TRH (although still increasing the rate of contraction) significantly decreased that of relaxation (by about 10%).

In summary, the above findings indicate that the TRH-induced increase in peak force (positive inotropic effect) is transient (Fig. 2), has a maximum at a particular (optimal) TRH concentration (Fig. 2), and is a result of apparently distinct effects of the hormone on cardiac contraction and relaxation (Fig. 3).

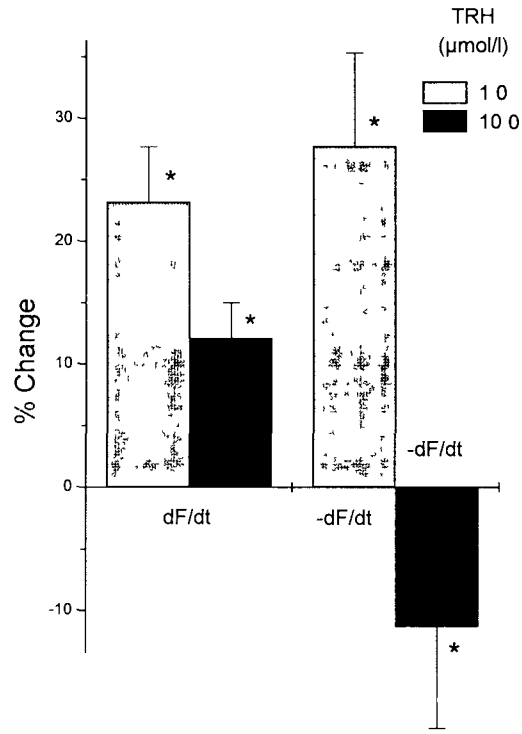

Figure 3. The influence of TRH on the rate of force development (dF/dt) and relaxation ($-dF/dt$) in isolated rat hearts. Data were obtained after 15 min exposure to 1 or 10 μmol/l TRH, and represent the average of at least 4 experiments. The asterisks show significant difference ($P < 0.05$) from time zero control values.

Discussion

The results reported herein indicate that TRH exerts a dual effect on cardiac muscle contractility, which most likely manifests itself through the binding of TRH to two types of TRH binding sites. A relatively slow binding of TRH to a higher-affinity

site appears to increase the rate of both contraction and relaxation, while a more rapid binding to a lower-affinity site seems to attenuate the rate of relaxation. At low concentrations, which appear to be physiologically more relevant (1), TRH is a positive inotrope, which likely results from the summation of its accelerating effect on both contraction and relaxation.

The dual effect of TRH on the performance of isolated hearts probably implies that TRH, through two separate binding sites, activates two distinct signal transduction pathways in the cardiac muscle cell. In various cell types, TRH has been shown to induce cytosolic Ca^{2+} transients by both the mobilization of the inositol 1,4,5-trisphosphate (Rebecchi and Gershengorn 1983; MacPhee and Drummond 1984; Mollard et al. 1990) cascade and the activation of Ca^{2+} entry into the cytoplasm via dihydropyridine-sensitive calcium channels (Enyeart et al. 1985; Wood and Schofield 1989). In pituitary cells, TRH has also been shown to increase cytosolic Ca^{2+} through a yet undetermined signaling pathway (Tashian et al. 1987) that might involve the mobilization of cyclic ADP-ribose, a newly discovered activator of the cardiac Ca^{2+} release channel (Mészáros et al. 1993). A TRH-induced increase in cytosolic Ca^{2+} through either of the above Ca^{2+} signaling pathways, all of which are documented to be functional in cardiac muscle cells (Brown and Jones 1986; Hess et al. 1986; Mikami et al. 1989; Kentish et al. 1990; Mészáros et al. 1993), could explain a TRH-induced increase in the rate of contraction, and thus an increased peak contractile force. However, a TRH-activated increase in the cytoplasmic Ca^{2+} through either pathways would certainly not be able to account for the TRH-induced decrease in the rate of relaxation, which was observed at higher TRH concentrations.

Another important point to note is that, contrary to the TRH-induced rise in cytosolic Ca^{2+} which occurs within seconds in many different types of cells (Tashian et al. 1987), both cardiac responses to TRH (i.e. the increase of contraction rate and the decrease in relaxation rate) developed rather slowly (when compared, for instance, to the adrenaline response, which fully develops within 30 s; not shown). Ravindra and Foster (1994) have recently shown that TRH-treatment increases the levels of Gq in GH3 pituitary cells, which effect of TRH had a similar time course to that we report in this study. Thus, it seems possible that TRH might exert its influence in the heart through the regulation of the levels of certain regulatory proteins such as G proteins. (In this respect, it is interesting to point out that we found a TRH-induced increase in the levels of ADP-ribosylation target proteins, most likely G proteins – unpublished observation).

TRH has been recently found to be expressed in rat hearts (Carnell et al. 1992; Lathrop et al. 1994). Furthermore, it has been also found that its expression in the heart is regulated by different steroid hormones such as glucocorticoids and testosterone (Lathrop et al. 1994). Thus, an intriguing possibility appears that the widely documented effects of these steroid hormones on cardiac performance might

become manifest through a TRH-mediated mechanism. This possibility as well as the identification of the TRH-activated signal transduction pathways relevant to the TRH-mediated alterations in cardiac contractility we describe here seem important to address in future studies.

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References

Beale J. S., White R. P., Huang S. P. (1977): EEG and blood pressure effects of TRH in rabbits. Neuropharmacology **16,** 499–506

Brown J. H., Jones L. G. (1986): Phosphoinositide metabolism in the heart. In: Phosphoinositides and Receptor Mechanisms. (Ed. J. W. Putney) pp. 245–270, Alan R. Liss

Carnell N. E., Feng P., Kim U. J., Wilber J. F. (1992): Preprothyrotropin-releasing hormone mRNA and TRH are present in the rat heart. Neuropeptides **22,** 209–212

Delbarre B., Senon D., Schmitt H. (1977): Effects of TRH and somatostatin on central regulation of blood pressure. Soc. Neurosci. Abstr. **3,** 19

Enyeart J., Aizawa T., Hinkle P. M. (1985): Dihydropyridine Ca^{2+} agonists: potent inhibitors of secretion from normal and transformed pituitary cells. Amer. J. Physiol. **248,** C510-C-519.

Eskay R. L., Long R. T., Palkovits M. (1983): Localization of immunoreactive thyrotropin releasing hormone in the lower brainstem of the rat. Brain Res. **277,** 159 162

Feuerstein G., Hassen A. H., Faden A. I. (1983): Cardiovascular and sympathetic modulation in brain nuclei of the rat. Peptides **4,** 617-620

Hess P., Lansman J. B., Tsien R. W. (1986): Calcium channel selectivity for divalent and monovalent cations. Voltage and concentration dependence of single channel current in ventricular heart cells. J. Gen. Physiol. **88,** 293–319

Holaday J. W., Faden A. I. (1983): Thyrotropin releasing hormone: Autonomic effects upon cardiovascular function in endotoxic shock. Regul. Pept. **7,** 111–125

Holaday J. W., D'Amato R. J., Faden A. I. (1981): Thyrotropin-releasing hormone improves cardiovascular endotoxic shock and hemorrhagic shock. Science **213,** 216–218

Kentish J. C., Barsotti R. J., Lea T. J., Mulligan I. P., Patel J. R., Ferenczi M. A. (1990): Calcium release from cardiac sarcoplasmic reticulum induced by photorelease of calcium or Ins(1,4,5)P3. Amer J. Physiol. **258,** H610 H615

Koivusalo F., Paakkari F. I., Leppaluoto J., Karppanen H. (1979): The effect of centrally administered TRH on blood pressure, heart rate and ventilation in rat. Acta Physiol. Scand. **106,** 83–86

Lathrop M., Sato T., Li Q. L., Feng P., Wilber J. F. (1994): Hormonal regulation of thyrotropin releasing hormone (TRH) gene expression in the rat heart. Proc. 76th Annu. Meeting Endocr. Soc., June 15–18, Anaheim CA, Abstract p. 232

MacPhee G. H., Drummond A. H. (1984): Thyrotropin-releasing hormone stimulates rapid breakdown of phophatidyl-inositol 4,5-biphosphate and phophatidyl-inositol 4-phosphate in GH3 pituitary tumor cells. Mol. Pharmacol. **25,** 193–200

Manaker S., Winokur A., Rostene W. H., Rainbow T. C. (1985): Autoradiographic localization of thyrotropin releasing hormone receptors in the rat central nervous system. J. Neurosci. **5,** 167–174

Mészáros L. G., Bak J., Chu A. (1993): Cyclic ADP-ribose as an endogenous regulator of the non-skeletal type ryanodine receptor Ca channel. Nature **364,** 76–79

Mikami A., Imoto K., Tanabe T., Niidome T., More Y., Takeshima H., Narumiya S., Numa S. (1989): Primary structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel. Nature **340,** 230–233

Mollard P., Duffy B., Vacher P., Barker J. L., Schlegel W. (1990): Thyrotropin-releasing hormone activates a [Ca]i-dependent K-current in GH3 pituitary cells via Ins(1,4,5) P3 sensitive and insensitive mechanisms. Biochem. J. **268,** 345–352

Morley J. E. (1979): Extrahypothalamic thyrotropin releasing hormone. Its distribution and its function. Life Sci. **25,** 1539–1550

Ravindra R., Foster K. A. (1994): Effect of thyrotropin-releasing hormone on Gq levels in GH3 cells. FASEB J. 8, Abstract p. 296

Rebecchi M. J., Gershengorn M. C. (1983): Thyroliberin stimulates rapid hydrolysis of phophatidylinositol 4,5-biphosphate by a phosphodiesterase in rat mammotropic pituitary cells. Biochem. J. **216,** 287–294

Tashian A. H., Heslop J. P., Berridge M. J. (1987): Subsecond and second changes in inositol polyphosphates in GH4C1 cells induced by thyrotopin-releasing hormone. Biochem. J. **243,** 305–308

Wood C. A., Schofield J. G. (1989): The effects of thyrotropin-releasing hormone and potassium depolarization on phosphatidyl-inositol metabolism and cytoplasmic calcium in bovine pituitary cells. Biochim. Biophys. Acta **1013,** 97 106

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