PTH-related protein and Type 1 PTH receptor mRNA expression in ventricular myocardial hypertrophy

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Background: The parathyroid hormone (PTH)-related protein (PTHrP) and Type 1 PTH receptor (PTH-1.R) bioregulation system exerts positive inotropic and chronotropic actions on myocardium *in vivo*, and it is implicated in cell–cell interactions, which can lead to myocardial hypertrophy. **Objective:** We analyzed the expression of PTHrP and PTH-1.R (mRNA and protein) in adult male Wistar rat ventricular myocardium after the induction of myocardial hypertrophy using aortic constricted and hyperthyroid models. **Materials & methods:** Expression of PTHrP and PTH-1.R mRNA was studied by semiquantitative reverse transcriptase polymerase chain reaction, while the nature of the polymerase chain reaction products was confirmed by direct DNA sequence. The expression of PTHrP and PTH-1.R in rat ventricular myocardium was confirmed using western blot and immunocytochemistry analysis. **Results:** The data showed that aortic banding-induced ventricular hypertrophy was associated with an increase in PTHrP mRNA expression, while hyperthyroid-induced ventricular hypertrophy was associated with PTH-1.R (mRNA and protein) overexpression in rat myocardium. **Conclusion:** Transcriptional changes of the PTHrP/PTH-1.R bioregulation system are differentially regulated in aortic constricting-induced and hyperthyroid-induced rat ventricular hypertrophy.

Parathyroid hormone (PTH)-related protein (PTHrP) was initially identified in the early 1980s as the humoral mediator of hypercalcemia associated with malignancy [1,2]. PTHrP primarily exerts its actions via the Type 1 PTH receptor (PTH-1.R), a member of the Class II G-coupled receptors (GCPRs). However, important PTHrP actions implicate other GCPR membrane receptors, such as PTH-2.R and zebrafish PTH-3.R, while the specific antiapoptotic actions of PTHrP have been attributed to PTHrP's actions as a transcription factor. The latter involves PTHrP phosphorylation, binding to β-importin and transportation to the nucleus [3–8].

PTHrP is expressed in various cells in the cardiovascular system, such as cardiac vascular smooth muscle cells, coronary endothelial cells and atrial cardiomyocytes; however, its actions are directed to cardiomyocytes, where PTH-1.R is expressed [2,9]. *In vivo*, PTHrP was demonstrated to improve myocardial function due to a positive inotropic and chronotropic action [10]. In addition, PTHrP actions can increase protein mass and p42-mitogen-activated protein kinase activity in myocardium while it can induce the re-expression of fetal-type proteins, such as creatine kinase BB in cardiomyocytes, *in vitro*. Furthermore, PTHrP activates protein kinase C in cardiomyocytes, leading to the acceleration of protein synthesis via an, as yet, unknown mechanism [11].

In a clinical setting, serum PTHrP concentration was shown to positively correlate with the degree of cardiac dysfunction of patients with chronic heart failure [12], suggesting that PTHrP participates in the pathophysiology of cardiac hypertrophy/heart failure in association with other peptides such as atrial natriuretic peptide (ANP) and arginine vasopressin [15]. Apparently, the coexpression of the calcium (PTHrP-dependent) and sodium (ANP-dependent) regulation systems in myocardium suggests that these two ion-regulating systems may participate in crucial pathophysiologic processes in human myocardium.

The experimental models currently employed for the study of myocardial hypertrophy include the chronic administration of the thyroxin (T4) (hyperthyroidism model) and the aortic constriction (AC) model. T4 actions on myocardium are apparently mediated via thyroid hormone receptor (TR) transcription activity, which results in volume overload-induced myocardial hypertrophy. Interestingly, several studies have indicated that T4-induced cardiac hypertrophy does not really affect the synthesis of contractile proteins in the rodent myocardium [13–15]. In contrast, in the AC model, myocardial hypertrophy is associated with an increase in protein synthesis [16], thus suggesting that the molecular mechanism(s) implicated in the induction of myocardial hypertrophy in these two experimental models are different.

Since little is known about the expression of PTHrP and PTH-1.R in hypertrophic myocardium, we analyzed the expression of PTHrP and PTH-1.R in rat ventricular myocardial hypertrophy, assessing PTHrP and PTH-1, mRNA and protein expression in the hyperthyroidism model (acute hyperthyroidism [a-HYP] model: T4 administration for 2 days; chronic hyperthyroidism [c-HYP] model: T4 administration for 7 days) and in the aortic-constriction model (surgically induced [AC] model). Our data suggest that AC-induced hypertrophy is associated with an increase in PTHrP mRNA expression, while hyperthyroid-induced hypertrophy (c-HYP model) was associated with an increase of PTH-1.R mRNA and protein expression. These data suggest that the PTHrP/PTH-1.R bioregulation system is possibly implicated in the pathophysiology of rat ventricular hypertrophy.

Materials & methods
Experimental procedure & animals
A total of 25 adult male Wistar rats, weighing between 280 and 330 g, were sacrificed for this study. Anesthesia was performed in these animals using intraperitoneal injection of ketamine hydrochoric acid (150 mg/kg). Animals were divided into five groups:

- a-HYP: 2 days of treatment with T4 (n = 5)
- c-HYP: 7 days of treatment with T4 (n = 5)
- AC (n = 5)
- Sham-operated animals (SOA) (n = 5)
- Controls (n = 5)

Hyperthyroidism was induced in rats by the administration of T4, which was dissolved in 99% ethanol by adding a small volume (20 µl) of 25% NaOH and diluted 33-fold by adding 0.9% NaCl in order to obtain a stock solution of 1 mg/ml. Before each injection, a fresh solution was prepared in 0.9% NaCl to a concentration of 50 µg T4/ml. T4 (25 µg/100 g body weight) was administered subcutaneously, once daily for up to 7 days (2 days for acute hyperthyroidism and 7 days for chronic hyperthyroidism). These treatments resulted in moderate hyperthyroidism, as was previously reported [17–19]. Control rats were given daily subcutaneous injections of normal saline for up to 7 days.

Abdominal aortic stenosis was produced using Weck hemoclips placed above the renal arteries near the diaphragm using modified Weck forceps. The diameter of the clips was calibrated to produce cardiac hypertrophy with a reasonably low mortality (~15% in our hands). This procedure can result in moderate myocardial hypertrophy, referred to as the AC model of myocardial hypertrophy, approximately 4 weeks after surgery [25]. Sham-operated animals have served as a control for this particular group. Cardiac hypertrophy was defined by the assessment of the left-ventricular weight (LVW) and total body weight (BW). Analysis was performed on absolute values of LVW and by calculating the ratio of LVW:BW (mg/g).

The protocol of our study was approved by the local Bioethics Committee and the animals were handled in accordance with the *Guide for the Care and Use of Laboratory Animals* [20].

RNA extraction–isolation & quantitative RT-PCR
Total RNA was extracted from myocardial ventricular tissue according to the procedure described by Chomczynski & Sacchi [21]. The concentration of RNA was estimated by measuring the optical density of the preparation at 260 nm. The ratio of the readings at 260 and 280 nm provided the estimate of the purity of the isolated RNAs. The purity of RNA is also estimated through electrophoresis on 1.1% ethidiumbromide-stained agarose gel. The isolated RNAs had ratios between 1.7 and 2.0. Reverse transcriptase (RT) reaction was carried out using:

- Depc-ddH$_2$O (Sigma, D05758)
- d-NTPs (10 Mm)
- SuperScript II RNase H
- Random primers – 0.3 µg/µl
- Min Oil (Sigma, M-5904) was added to minimize evaporation and cross contamination

The reaction was incubated at 42°C for 50 min and inactivated at 70°C for 20 min.

Semiquantitative polymerase chain reaction (PCR) was performed for RT products, using:

- Depc-ddH$_2$O
- d-NTP's (10Mm)
- Taq. polymerase (5 U/µl)
- Primers (10 Mm) of both rat PTHrP and PTH-1.R
- Min. oil

The primers were designed from the genomic sequence of the rat PTHrP and PTH-1.R gene regions (genebank accession numbers NM_012636 and L19475, respectively). The amplified products were then checked on 1.8% ethidiumbromide-stained agarose gel. The cycle parameters for the PTHrP target fragments were:

- One cycle at 95°C for 5 min

- 35 cycles at 95°C for 30 s, 60°C for 30 s and 72°C for 30 s

- Final cycle at 72°C for 5 min

The cycle parameters for the PTH-1.R target fragment were:

- One cycle at 95°C for 5 min

- 36 cycles at 95°C for 30 s, 54°C for 30 s and 72°C for 30 s

- Final cycle at 72°C for 5 min

Expression of PTHrP and PTH-1.R mRNA was investigated through quantitative RT-PCR technique, using as an internal standard the 18S ribosomal RNA. The image analyzing system Kodak EDAS 290 Electrophoresis Documentation and Analysis System Software was used for the measurements

Identifying the PCR product

Direct sequencing of PCR products was performed in 20 l volumes using dye-labelled dideoxy terminator chemistry, as published previously [22,23], using the ABI Prism 373 analysis system. Each reaction contained 8 l Dye Terminator Ready Reaction Mix (Dye terminator cycle sequencing ready reaction kit with Amplitag FS, Catalog no. 402079, ABI Biosystems), 0.2 to 0.4 ng of each PCR product and 0.25 l of each specified primer. The cycling conditions were set at 24 cycles at 96°C for 30 s, 50°C for 15 s and 60°C for 4 min. The sequencing reaction products were then ethanol precipitated and loaded on a model 373A automated DNA Sequencer for analysis.

Western analysis & immunohistochemistry

Frozen tissue (0.15 g) was homogenized in ice-cold Tris-Buffer (100 mM; pH 6.8) and the resulted homogenate was filtrated twice. Protein concentration was determined by bicinchoninic acid method using bovine serum albumin as a standard. Protein samples were diluted in equivalent quantity of loading buffer (Tris-HCl 0.5 M; pH: 6.8, glycerol, sodium dodecyl sulphate 10%, β-mercaptethanol and bromophenol blue 0.5%). Equal amounts of protein extracts (30 μg) were resolved by 12.5% sodium dodecyl sulphate–polyacrylamide gel electrophoresis gel and transferred onto nitrocellulose filters. Blots were incubated with nonfat milk 5% in phosphate-buffered saline (PBS) for 1 h, and then incubated overnight at 4°C with mouse monoclonal antibody against the amino acid residues 38 to 64 of PTHrP (antibody GF08, Oncogene Research products) and, second, with an antimouse immunoglobulin (Ig) antibody coupled to alkaline phosphatase [24]. PTH-1.R expression was assessed using rabbit polyclonal antibody against PTH receptor peptide VII, CLVDADDVFTKEEQI (PRB-635P, Eurogentec) and second with an antirabbit IgG-HRP conjucated antibody.

Immunohistochemistry was performed by slicing the formalin-fixed (using 10% buffered formalin for 48 h) and paraffin-embedded tissues. Consecutive sections were cut with microtome using slides coated poly-L-lysine. The tissue sections were deparaffinized with xylene three times and rehydrated in descending concentrations of ethanol. The sections were then washed with dd-H$_2$0 for 3 min, and heated at 95°C, for 5 min in a 10 mM solution of sodium citrate (pH 6). After having been embedded in ddH$_2$0 for 3 min, the sections were washed twice with cold PBS (pH 7.4), incubated in 3% hydrogen peroxide in methanol for 10 min, washed with PBS and then treated with blocking serum for 10 min at room temperature. The tissue sections were incubated overnight at 4°C with primary antibody for PTHrP (mouse monoclonal IgG; antibody GF08) and PTH.1-R at a dilution of 1 in 10 and 1 in 25, respectively. As negative controls were used, there were tissue sections that received no primary antibody. After another wash with PBS, the tissue sections were treated with the secondary antibody (antimouse IgG conjugated with horseradish peroxidase) in PBS for 60 min at room temperature. Color development was visualized with 3,3-diaminobenzidine (DAB) substrate system. The slides were counterstained with hematoxyline and mounted for examination.

Statistics

Values are presented as the mean + standard error of the mean value (X + SE). An unpaired t-test was used for differences between groups (p < 0.05 was considered significant). In addition, we used the one-way factorial analysis of variants (ANOVA) (p < 0.05) when necessary.

Results
Defining cardiac hypertrophy

Analysis of LVW and LVW to BW ratio in the a-HYP model showed that these parameters did not differ from those measured in controls, thereby suggesting that 2 days' administration of T4 does not really produce morphometric evidence of

Figure 1. Expression of PTHrP and PTH-1.R detected in normal rat ventricular myocardium as analyzed by RT-PCR.

Both RT-PCR products where analyzed by DNA sequence, confirming the nature of PTHrP and PTH-1.R. Furthermore, it is indicated the linear way of increase of both PTHrP and PTH.1-R by increasing numbers of PCR cycle. Detection of PTHrP (48 kDa) and PTH.1-R (66 kDa), in rat ventricular myocardium as assessed by western blot analysis using mouse monoclonal antibody against the aminoacid residues 38 to 64 of PTHrP and rabbit polyclonal antibody against PTH receptor peptide VII, CLVDADDVFTKEEQI. Methodological details, including the RT-PCR method are described in the Materials & method section.
PCR: Polymerase chain reaction; PTH-1.R: Type 1 PTH receptor;
PTHrP: Parathyroid hormone-related protein; RT-PCR: Reverse transcription polymerase chain reaction.

cardiac hypertrophy [17–19]. Nevertheless 2 days' T4 administration can serve as an *in vivo* model for studying the direct actions of thyroid hormones (via TR transactivation) on PTHrP and PTH-1.R

genes in rat myocardium before the development of morphometric evidence of myocardial hypertrophy in the volume-overload model (LVW/a-HYP = 882.6 + 33.1 vs. C = 887.8 + 33.5 mg; [X + SE, p > 0.05]; LVW/BW: a-HYP = 2.6 + 0.10; C = 2.5 + 0.06 mg/g [p + SE, p > 0.05]).

However chronic T4 administration (c-HYP) did produce the experimental evidence of myocardial hypertrophy, as noted by the significant increase of the LVW and LVW/BW ratio in c-HYP animals (LVW/c-HYP = 1108 + 46.8 vs. C = 872.2 + 23.3 mg; [X + SE, p < 0.05]; LVW/BW: c-HYP = 3.1 + 0.09 vs. C = 2.5 + 0.10 mg/g [X + SE, p < 0.05]).

In addition, significant increases of the LVW and LVW/BW ratio were documented in animals subjected to AC as compared with sham-operated animals (LVW: AC = 988.2 + 23.8 vs. sham-operated animals = 909.5 + 25.5; mg [X + SE, p < 0.05]; LVW/BW: AC = 2.16 + 0.06 vs. sham-operated animals = 1.83 + 0.04 mg/g [X + SE, p < 0.05]).

Analysis of PTHrP & PTH-1.R expression in normal rat ventricular myocardium
Our specific primers depicted a 268 bp PCR and a 498 bp band, corresponding to the expected sizes of PCR products amplified by our PTHrP- and PTH-1.R-specific primers, respectively. The nature of these PCR products was defined by direct DNA sequence **(Figure 1a & b)**. Furthermore, PTHrP and PTH-1.R expression was detected at the protein level using western analysis **(Figure 1a & b)** and immunocytochemistry **(Figure 2)**, which actually revealed that PTHrP is mostly expressed by the coronary endothelial cells and PTH-1.R by the cardiomyocytes of the rat ventricular myocardium.

PTHrP & PTH-1.R mRNA expression in rat ventricular myocardium in hyperthyroidism
Total RNA from the ventricular myocardium of experimental and control animals were analyzed by semiquantitative RT-PCR method. Relative PTHrP mRNA expression did not show any significant differences between either c-HYP or a-HYP versus controls **(Figure 3a)**. However, PTH-1.R mRNA expression was found as increased in the c-HYP group, compared with controls (increase of relative PTH-1.R expression; p < 0.05). These data suggest that an increase of the PTH-1.R mRNA expression is associated with hyperthyroid-induced myocardial hypertrophy; however, not with AC-induced hypertrophy. Notably, 2 days' treatment with T4

Figure 2. Example of the expression of PTHrP and PTH-1.R detected in ventricular myocardium as analyzed by immunohistochemistry.
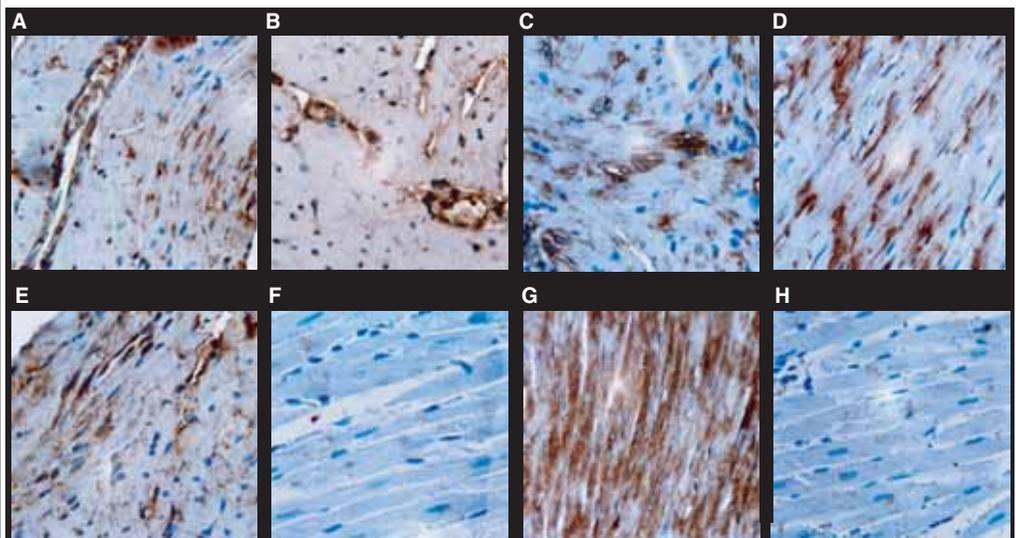
(A) Control: PTHrP expression in rat ventricular myocardium of control animals. (B) Aortic constriction: PTHrP expression in rat ventricular hypertrophy of animals in the aortic constriction model. (E) PTHrP expression in chronic hyperthyroid rat ventricular myocardium model. (F) Negative control. (C) Control: PTH-1.R expression in rat ventricular myocardium of control animals. (D) Aortic constriction: PTH-1.R expression in rat ventricular hypertrophy of animals in the aortic constriction model. (G) PTH-1.R expression in chronic hyperthyroid rat ventricular myocardium model. (H) Negative control.
PTH-1.R: Type 1 PTH receptor; PTHrP: Parathyroid hormone-related protein.

did not significantly influence the relative PTHrP and PTH-1.R mRNA expression in rat ventricular myocardium, suggesting that TR trans-activation does not alter directly *PTHrP* and *PTH-1.R* gene expression, *in vivo*.

PTHrP & PTH-1.R immunohistochemical analysis in the rat ventricular myocardium in hyperthyroidism model
PTHrP and PTH-1.R protein was localized in control and hyperthyroid myocardium by immunohistochemical analysis. PTHrP expression was detected mainly in coronary endothelial cells while PTH-1.R in ventricular cardiomyocytes. In fact the PTH-1.R had detectable greater expression in the c-HYP group, compared with controls (Figure 2). These data are in line with the expression of PTHrP and PTH-1.R mRNA expression, suggesting that hyperthyroid-induced hypertrophy is associated with an overexpression of both mRNA and protein PTH-1.R expression.

PTHrP & PTH-1.R mRNA expression in rat ventricular myocardium after AC
Relative PTHrP mRNA expression in AC animals versus sham-operated animals revealed that AC-induced ventricular hypertrophy in rat ventricular myocardium was associated with an increase of PTHrP mRNA expression (p < 0.05); however, not with PTH-1.R mRNA expression (Figure 4a and 4b).

PTHrP & PTH-1.R immunohistochemical analysis in rat ventricular myocardium after AC
PTHrP and PTH-1.R protein was localized in control and hyperthyroid myocardium by immunohistochemical analysis. PTHrP expression was detected mainly in coronary endothelial cells, while PTH-1.R was mainly found in ventricular cardiomyocytes (Figure 2). Our results didn't show any appreciable change in the expression of PTHrP and PTH-1.R protein between control and aortic myocardium. However, it is important to note that the semi-quantitative nature of immunohistochemistry is unable to depict small, yet significant, changes in proteins.

Discussion
Cardiac hypertrophy represents an important risk factor for cardiovascular morbidity and mortality, which is directly associated with the increasing frequency of ventricular arrhythmias

Figure 3. Example of the relative expression of PTHrP and PTH-1.R mRNA in rat ventricular hypertrophy experimentally induced by volume overload.
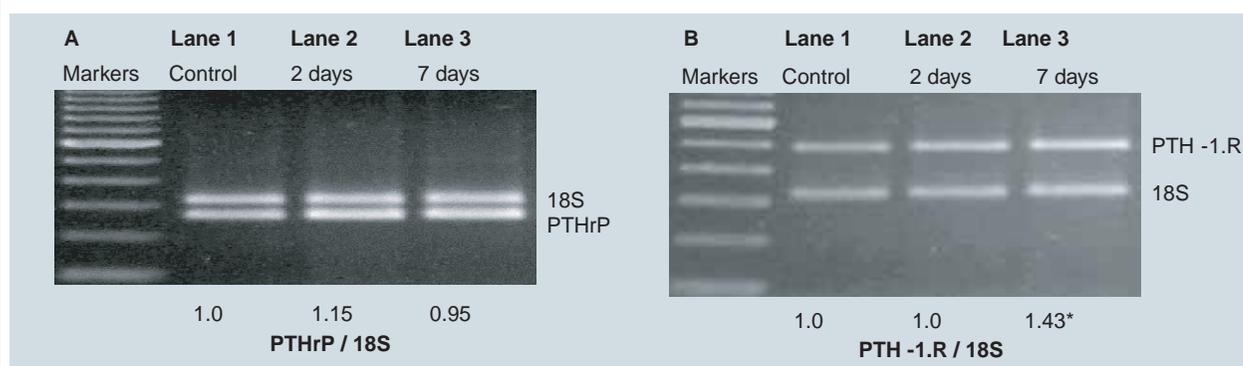
(A) Lane 1 – markers: molecular-weight markers. Lane 2 – control: PTHrP expression in rat ventricular myocardium of control animals. Lane 3 – 2 days: PTHrP expression in acute hyperthyroid rat ventricular myocardium model. Lane 4 – PTHrP expression in chronic hyperthyroid rat ventricular myocardium model. (B) Lane 1 – markers: molecular-weight markers. Lane 2 – control: PTH-1.R expression in rat ventricular myocardium of control animals. Lane 3 – 2 days: PTH-1.R expression in acute hyperthyroid rat ventricular myocardium model. Lane 4 – 7 days: PTH-1.R expression in chronic hyperthyroid rat ventricular myocardium model.

*$p < 0.05$. Relative expression was assessed by referring to the 18S ribosomal RNA expression, as described in Materials & methods section. PTH-1.R: Type 1 PTH receptor; PTHrP: Parathyroid hormone-related protein.

and negative impact on the overall survival. Different causes can result in myocardial hypertrophy; however, hypertrophy develops in response to either pressure or volume overload. Pressure overload results in concentric hypertrophy, which is characterized by the lateral expansion of cardiomyocytes with the addition of new sarcomeres in parallel. Consequently, the wall thickness is increased with minor chamber enlargement, resulting in an increase in the mass to volume ratio, which attempts to normalize myocardial wall stress. In contrast, volume overload results in cardiomyocyte lengthening, which occurs with the addition of new sarcomeres in series. Thus, ventricular dilatation is accompanied by proportional increase in wall thickness [25–29].

Recently, expression of the PTHrP/PTH-1.R bioregulation system was identified in cell populations of myocardium and vascular smooth muscle cells [2,9]. Consequently, our study was designed to determine whether changes of the PTHrP/PTH-1.R transcription accompany the development of myocardial hypertrophy in rat ventricular myocardium. In addition, we explored whether these putative transcriptional changes of the PTHrP/PTH-1.R gene are associated with myocardial hypertrophy *per se* as an end result or are dependent on the cause, which mediates the development of rat ventricular hypertrophy (AC- vs. hyperthyroid-induced myocardial hypertrophy). The efficacy of these *in vivo* models to induce myocardial hypertrophy was confirmed by the analysis of the LVW and LVW/BW ratio in experimental versus control or sham-operated animals. Our data revealed that aortic constriction-induced hypertrophy is associated with overexpression of PTHrP mRNA whereas hyperthroid-induced hypertrophy is associated with overexpression of PTH-1.R mRNA and protein expression. These data are in line with previous studies, which have suggested that PTHrP bioregulation system is regulated by the mechanical stress in coronary arteries [30]. In addition, previous data showed that PTHrP and PTH-1.R expression is restricted to a specific cell population of the rat ventricular myocardium. Indeed, our immunohistochemistry data support the notion that ventricular cardiomyocytes express PTH-1.R, thereby being the main targets for PTHrP actions in rat ventricular myocardium, while the coronary endothelial cells express mostly expressed PTHrP, representing the main source for PTHrP production in rat ventricular myocardium.

Therefore, it is conceivable that coronary endothelial cells enhance PTHrP mRNA expression during AC-induced hypertrophy, while the cardiomyocytes become more sensitive to PTHrP actions (overexpressing PTH-1.R) during hyperthyroid-induced hypertrophy in rat ventricular myocardium. Whether this represents a similar pattern of PTH/PTH-1.R expression in the volume-overload and pressure-overload model

Figure 4. Example of the relative expression of PTHrP and PTH-1.R mRNA in rat ventricular hypertrophy experimentally induced by aortic stenosis model.
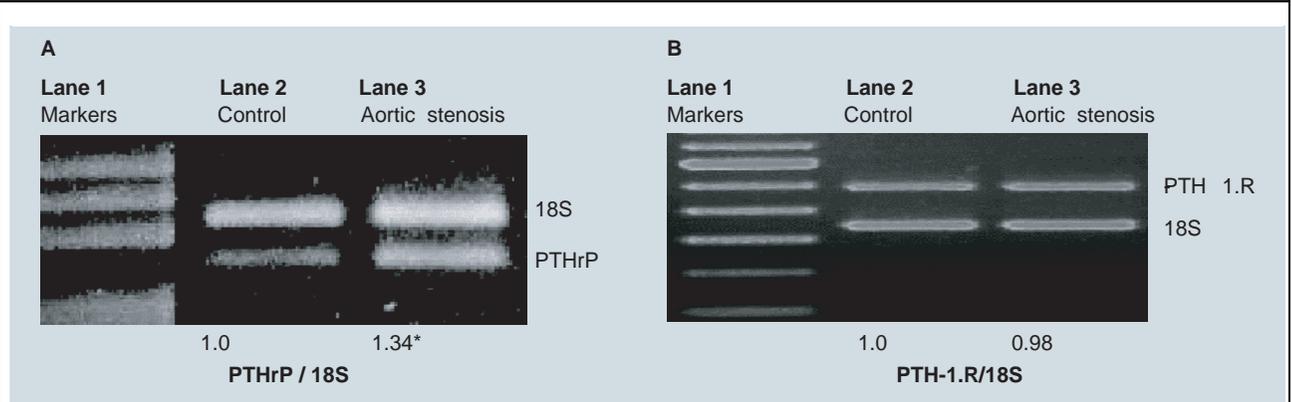
(A) Lane 1 – control: molecular-weight markers. Lane 2 – control: PTHrP expression in rat ventricular myocardium of control animals. Lane 3 –aortic stenosis: PTHrP expression in rat ventricular hypertrophy of animals in the aortic constriction model. (B) Lane 1 – markers: molecular weight markers. Lane 2 – control: PTH-1.R expression in rat ventricular myocardium of control animals. Lane 3 – aortic stenosis: PTH-1.R expression in rat ventricular hypertrophy of animals in the aortic constriction model.

*$p < 0.05$ Relative expression of PTHrP and PTH-1.R mRNA was assessed by referring to 18S ribosomal RNA, as described in the Materials & Methods section.

PTH-1.R: Type 1 PTH receptor; PTHrP: Parathyroid hormone-related protein.

requires further investigation. Consequently, it is possible that a cause-related increase of the transcription of either the *PTHrP* or *PTH-1.R* gene in the development of rat ventricular hypertrophy occurs.

Indeed, previous studies have shown evidence that the PTHrP/PTH-1.R bioregulation system may be part of pathophysiologic processes in cardiovascular diseases. Such studies have documented that the expression of the PTHrP in vascular smooth muscle cells is regulated by vasoconstrictors, such as norepinephrine, endothelin 1, angiotensin II, serotonin, bradykinin and thrombin, and by mechanical stress such as mechanical distention of the vascular wall [29–31]. Other experimental data have shown that PTHrP is upregulated after balloon angioplasty and by the increasing arterial blood pressure [32]. In addition, mechanical stress induces the expression of PTHrP in vascular smooth muscle cells while alterations in blood flow promote a mechanosensitive release of PTHrP from coronary endothelial cells [33]. Interestingly, tumor necrosis factor (TNF)-α and interleukin (IL)-1β can enhance PTHrP production in a time- and dose-dependent manner, *in vitro* [34]. Moreover, a recent study using endothelial cells from rat ventricular myocardium reported that transforming growth factor (TGF)-β1 affects the PTHrP production [41], thus reinforcing the concept that PTHrP may be implicated among other local growth factors in myocardial hypertrophy [35].

Conclusion

In this context, our data are in line with previous reports concerning the possible role of the PTHrP/PTH-1.R bioregulation system in cardiovascular diseases, suggesting that interactions between local bioactive substances, such as growth factors and vasoactive substances with the PTHrP/PTH-1.R bioregulation system may have a specific role in the development of myocardial hypertrophy. The specific role of such bioactive substances can vary according to the causative pathological mechanism implicated in myocardial hypertrophy.

Highlights

- Aortic constriction-induced ventricular hypertrophy is associated with parathyroid hormone (PTH)-related protein overexpression in rat ventricular myocardium.
- Hyperthyroid-induced ventricular hypertrophy is associated with PTH-1 receptor overexpression in rat ventricular myocardium.

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