

The PTHrP/PTH.1-R Bioregulation System in Cardiac Hypertrophy: Possible Therapeutic Implications

ANTONIS HALAPAS¹, EVANTHIA DIAMANTI-KANDARAKIS²,
DEMETRIOS KREMASTINOS³ and MICHAEL KOUTSILIERIS¹

¹Department of Experimental Physiology, ²Endocrine Section, First Department of Internal Medicine and

³Department of Cardiology, University of Athens, Medical School, Greece

Abstract. Myocardial hypertrophy is associated with specific histological changes, which are representative of the cardiomyocytes cellular response to overload. Evidently, transcriptional modulation of specific genes is implicated in myocardial hypertrophy. Recently, the parathyroid hormone-related protein (PTHrP)/PTH-1 receptor (PTH-1.R) bioregulation system was shown to participate in specific regulatory processes of the cardiomyocyte function and proliferation, which can be related to cardiac hypertrophy and heart failure. We review the literature on the pathophysiology of cardiac hypertrophy vis-a-vis the role of PTHrP/PTH-1.R system as a good therapeutic target.

Heart failure is defined as the inability of the heart to cope with the demands of the peripheral system (1). The onset of cardiac failure defines a survival of 1.5-3.5 years (2, 3). Moreover, the single most powerful predictor for the development of heart failure is the presence of left ventricular (LV) hypertrophy (4).

Heart failure (HF) is currently viewed as a complex progressive process rather than a single event (5). Notable, LV dysfunction is necessary but not sufficient for the development of heart failure. Thus, the development and progression of HF is best described as a multistep process resulting in a gradual systolic dysfunction. Furthermore, diastolic dysfunction is also an important contributor to HF (6).

Correspondence to: Michael Koutsilieris, MD, Ph.D, Professor & Director, Department of Experimental Physiology, University of Athens, Medical School, 75 Micras Asias Goudi Athens, 115 27, Greece. Tel: +30 210 7462597, Fax: +30 210 7462571, e-mail: mkouts@medscape.com

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Stage I of HF (Preserved function): Numerous adaptive compensatory mechanisms are activated in the myocardium, and, as a result, the overall cardiac function is preserved by these mechanisms.

Stage II of HF (Compensated dysfunction): This is believed to represent a maladaptive phase. The type of injury and the initially adaptive compensatory mechanisms interact to bring about the progressive deterioration of LV function. The structure and shape of the LV are altered and the global cardiac function is abnormal. Further maladaptive mechanisms are activated and heart failure is fully established. Unfortunately, the transition from compensated LV dysfunction to overt HF is not well understood.

Stage III of HF (Decompensated overt heart failure): In this case the LV structure, shape and size is abnormal and characterized by wall thinning, LV sphericity and profound dilatation. Global function is often severely impaired. The duration of this phase, as well as the transition period from one phase to the next, depends mainly on the type of the hypertrophic stimuli.

At the cellular level cardiomyocyte hypertrophy is characterized by an increase in cell size, enhanced protein synthesis and heightened organization of the sarcomere. Two different hypertrophic phenotypes can be distinguished: (a) an eccentric hypertrophic phenotype, due to volume overload or prior infraction, characterized by the addition of sarcomeres in series and longitudinal cell growth (7); and (b) concentric hypertrophy due to pressure overload, which is characterized by the lateral expansion of myocytes from the addition of new sarcomeres in parallel (9).

Interestingly, cardiomyocyte replication occurs in normal myocardium and is increased in diseased myocardium, particularly in the border zone between the infarcted and viable myocardium (9-11). Indeed, a small proportion of cardiomyocytes retain the ability to reenter the cell cycle,

becoming particularly prolific when the myocardium is injured and cell losses occur. Thus, the generation of new myocytes is likely a major determinant of cardiac remodeling in the failing heart (12).

At the molecular level, volume-overload and pressure-overload evoke differential regulation of gene transcription, resulting in a distinct cardiomyocyte response (13). Indeed, marked differences in the expression levels of β -myosin, α -skeletal actin and SERCA-2a were observed in pressure overload-induced hypertrophy relative to volume overload (15). This heterogeneity at the molecular level presumably reflects differences in the way in which each of the two types of hemodynamic overload activate intracellular signaling pathways that ultimately result in gene reprogramming.

Certain biochemical pathways (such as the PI3K/Akt pathway) are involved in the development of an adaptive form of cardiac hypertrophy that does not show decompensation in the long term (15). In contrast, other biochemical pathways (such as that of calcineurin) determine a maladaptive cardiac hypertrophy and HF (16). It has been documented that by altering the expression of specific peptide hormones, growth factors and cytokines, one can activate specific histological features of the hypertrophic response *in vitro* (17, 18) (Figure 1).

Thus, the signal for cardiac hypertrophy is mediated by a complex cascade of signaling systems within the cardiomyocytes resulting in gene reprogramming. Therefore, it is essential to identify molecular events involved in the hypertrophic process and differences in the signaling systems that promote pathological hypertrophy. We present a brief summary of the current knowledge on the possible relation of the PTHrP/PTH-1.R bioregulation system with the pathophysiology of cardiac hypertrophy.

The PTHrP / PTH-1-R Bioregulation System and Pathophysiology of the Myocardium

The parathyroid hormone related peptide (PTHrP) is a single copy gene with close homology to parathyroid hormone (PTH). The protein was identified in the early 1980's and the cloning of the PTHrP cDNA from a human squamous cell lung cancer cell line was reported in 1987 (19). PTHrP is expressed in a wide range of physiological tissues during fetal development and the adult life of rat, mouse, chicken and humans (20). In human tissues, PTHrP transcripts are expressed in the skin, bone marrow, fetal liver, lung, spleen, smooth muscle cells, gastric mucosa, pituitary, adrenal, thyroid and parathyroid glands, blood vessels, lactating mammary tissue and the central nervous system (21, 22). The physiological functions of PTHrP are carried out locally and include the regulation of smooth muscle tone, differentiation and proliferation, transepithelial calcium transport, tissue and organ development (22).

However, systemic effects of PTHrP were initially reported in malignancies causing the common paraneoplastic syndrome of humoral hypercalcemia malignancy (HHM) (23, 24). The wide physiological importance of PTHrP is suggested by the fact that neonatal mice with homozygous ablation of the gene encoding either PTHrP or the PTH/PTHrP receptor die at, or just before, birth and exhibit widespread skeletal abnormalities (25).

The human PTHrP gene is highly conserved among species. It is localized in the short arm of chromosome 12, spans approximately 15 kb of genomic DNA and consists of 9 exons (25), from which only 2, exon V and VI, occur in all transcriptional products. The 9 exons expressing the human PTHrP gene undergo alternative splicing of their mRNA to produce three different-sized initial translation products that are controlled by three different promoters, P1, P2 and P3 (26, 27). While P1 and P3 are TATA promoters, whole P2 is a GC-rich promoter (27, 28). No evidence for alternatively spliced forms of PTHrP in rat, mouse or chicken mRNA has been demonstrated to date.

The full length of the PTHrP mRNA translational product is a precursor protein processed into several smaller peptides that have diverse biological functions. These functions are determined by the modifications of the precursor PTHrP, which include amidation, glycosylation or other processing steps affecting their final structures. In the three forms of the human PTHrP, the primary sequence of amino acid residues 1-139, 1-141, and 1-173 vary only in their C-terminus (26, 27). Eight of the first 13 amino acids of all three forms of the PTHrP peptide are common with PTH but in the 13-34 region the homology decreases and only 3 of the amino acids are identical. Beyond amino acid residue 34, there is no recognizable amino acid sequence similarity between PTH and PTHrP. It is noteworthy that in both PTH and PTHrP the 15-34 region is the principal PTH/PTHrP binding domain (26-29). Although PTH and PTHrP are not similar in amino-acid sequence, they are almost similar in their secondary and tertiary structure. Each of the mature N-terminal, mid region and C-terminal secretory forms of PTHrP generated have their own physiological functions and possibly their own receptors. The type 1 receptor (PTH-1.R) binds both the PTH 1-34 and the PTHrP 1-36 regions and is the only known receptor to do so (29). In the mid-region, there is a sequence of basic amino acids (88-106 region) that is a nuclear localization signal (NLS). Furthermore, recently a second basic amino-acid sequence in 147-150 has been found to be an important intracrine regulator in chondrocytes. The complexity of the PTHrP contrasts with that of the PTH gene, which is simpler in structure and regulation (29-32).

The PTH-1R is a class II G-Coupled Protein Receptor (GPCR) with seven transmembrane segments. It binds both the PTHrP and PTH (with indistinguishable affinity) while

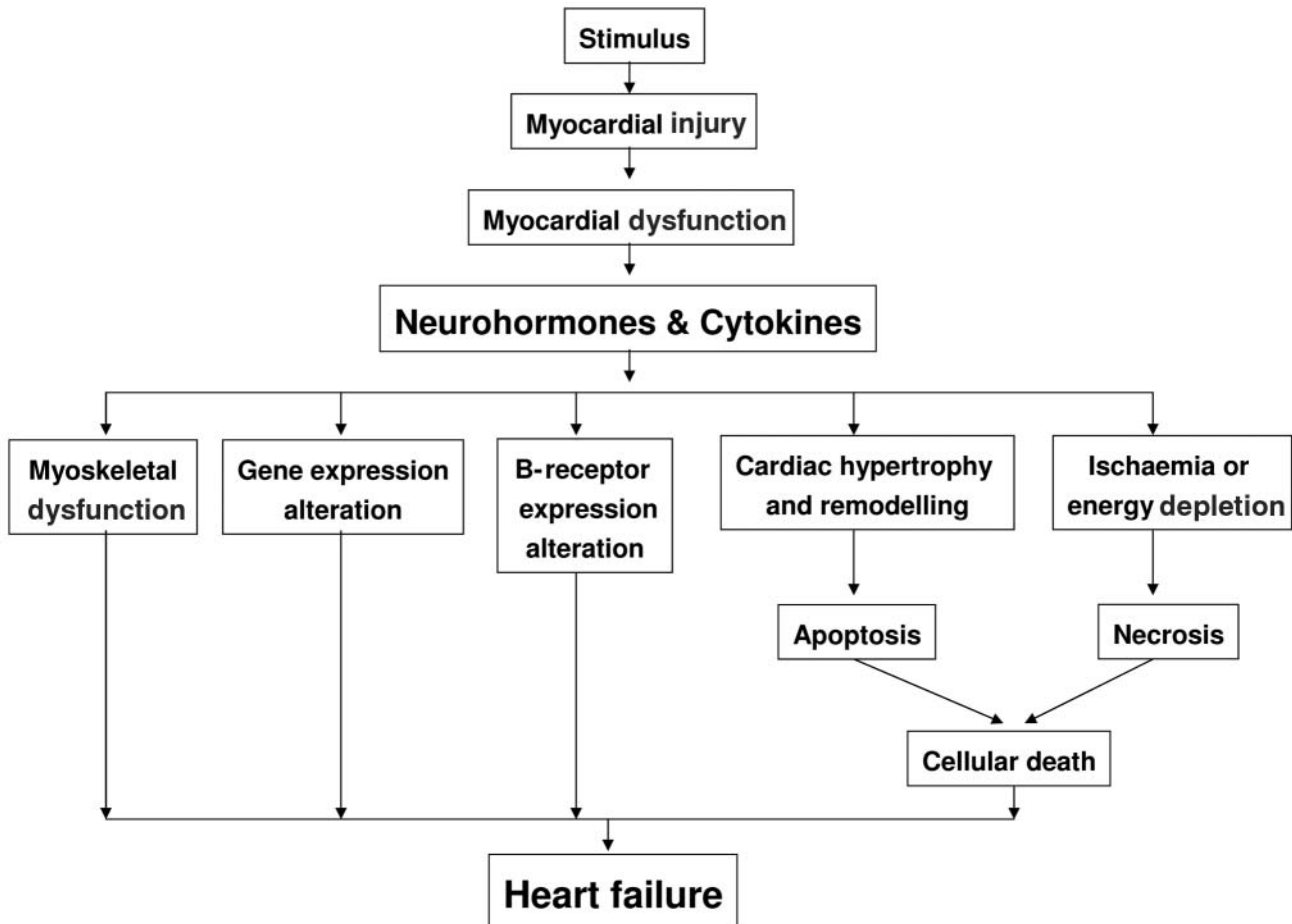


Figure 1. An outline of the pathophysiological aspects in the development of cardiac hypertrophy and heart failure. The role of the neurohormonal system and cytokines.

the type 2 PTH receptor (PTH-2.R), with a 51% homology with PTH-1.R, responds fully to PTH but exhibits very low affinity to PTHrP (29, 30, 33). Furthermore, in rats PTH-2.R does not respond either to PTH or to PTHrP. This observation led to the belief that another factor selectively activates PTH-2.R receptor. Experimental data revealed that a 39 amino acid peptide, TIP39, activates PTH-2.R both in rats and humans (34). A new member of the PTH-Receptor family was discovered in zebra-fish, z-PTH-3.R. The z-PTH-3.R exhibits 61% homology to z-PTH-1R but less homology to z-PTH-2R and responds fully to PTHrP but exhibits very low affinity to PTH (35).

Activation of the PTH/PTHrP receptor by either PTH or PTHrP results in increased Ca^{2+} release from bone and enhanced reabsorption of Ca^{2+} in the kidney (30), thereby playing a fundamental role in the regulation of mineral ion homeostasis (28, 29). Following binding of the ligand, the receptor is coupled strongly to the AC-PKA signaling pathway and less strongly to the PLC-PKC signaling pathway.

Moreover, the receptor transduces the signal *via* the activation of the Gs and Gq membrane proteins in bone and kidney, whereas in the smooth muscle its responses are mainly mediated through the AC-PKA signaling pathway (36-38). The proposed mechanism for the ligand-receptor interaction raises two crucial points: i) the interaction between the C-terminal domain of the ligand and the N-terminal domain of the receptor contributes primarily to binding affinity, and ii) an interaction between the N-terminal portion of the ligand and the juxta-membrane region of the receptor contributes to the signal transduction. Experimental data (36) show the PTH-1.R undergoes agonist-induced endocytosis. This process involves the interaction of the occupied PTH-1.R with members of the arrestin family of proteins at the cell membrane, followed by the targeting of the agonist-PTH-1.R-arrestin complex to clathrin coated pits, ending with the subsequent internalization of the complex. After internalization, the PTH-1.R is recycled to the cell membrane in a re-sensitized, functional state (37-39).

In contrast to pathological conditions, such as humoral hypercalcaemia malignancy (HHM) where PTHrP functions as an endocrine hormone, under normal circumstances PTHrP functions in a paracrine/autocrine manner. Considerable evidence indicates that PTHrP exerts an additional signaling not by extracellular action but by translocation to the nucleus *via* targeting sequences where it influences nuclear function. The consequences of this intracrine mode of action are not yet well characterized but they may modulate processes of vital importance to the cell such as the inhibition of apoptosis (37-39). The intracrine growth regulatory effects of PTHrP were studied in a prostate cancer (PC-3) and a breast cancer (MCF-7) cell line (40-43). Mutations of the NLS abrogated the proliferative effects and nuclear translocation of PTHrP, indicating the involvement of an intracrine pathway in the mode of action of PTHrP. Cells overexpressing wild type PTHrP were enriched in the G₂/M cell cycle phase, protected from serum-starvation induced apoptosis and showed higher ratios of the anti-apoptotic proteins bcl-2 and bcl-x_L to bax than cells overexpressing NLS-mutated PTHrP. These studies indicate that an intact NLS is required for the PTHrP-mediated protective effects against apoptosis (40-43). Several studies showed that the specific import of PTHrP into the nucleus is accomplished through binding to importin β which, together with the protein Ran, is able to mediate the efficient nuclear accumulation of PTHrP. The nuclear import of PTHrP may also occur *via* an intracrine pathway of a cell expressing PTHrP or in a target cell, as a result of the PTHrP binding to the PTH-1.R and internalization of this complex (44). Alternatively, PTHrP may influence nuclear function through binding to an intracellular PTH-1.R. This PTH-1.R has been localized in the cell nucleus suggesting that it may regulate nuclear events, either by influencing the cytoskeleton or affecting gene expression directly (45).

Some of the functions attributed to PTHrP are: PTH-like calciotropic function, myorelaxant function, neuroendocrine peptide activity, growth factor action, and developmental regulatory function (26). One common feature of the multiple functions of PTHrP, in both normal and malignant tissues, is that they are growth and proliferation related. Factors implicated in the regulation of PTHrP expression include cytokines, growth factors, and hormones. Those shown to up-regulate PTHrP include epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), interleukin-6 (IL-6), transforming growth factor betas (TGF β s) and estradiol (E2), while those involved in down-regulating PTHrP expression include androgens and dexamethasone (20). These data suggest that PTHrP may interact with oncogenes. PTHrP transcription was reported as a consequence of the ras / src oncogene activation and as a target of the Bcl-2 anti-apoptotic gene (45-48). The complex regulation of PTHrP expression

also involves glucocorticoid receptor (GR) (49) and the vitamin D receptor (Vit.D3.R) (50).

Recently, expression of the PTHrP/PTH-1.R bioregulation system was identified in cell populations of myocardium and vascular smooth muscle. PTHrP is expressed in cardiac vascular smooth muscle cells, coronary endothelial cells and atrial cardiomyocytes, while PTH-1.R is mainly expressed in cardiomyocytes, thereby acting as a paracrine factor in the myocardium (39, 51, 52). In addition, experimental data using immunohistochemistry supported the notion that ventricular cardiomyocytes express PTH-1.R, thereby being the main targets for PTHrP action in rat myocardium, while coronary endothelial cells mostly expressed PTHrP, representing the main source for PTHrP production in rat ventricular myocardium (53).

In vivo, PTHrP was shown to improve myocardial function due to a positive inotropic and chronotropic action. In ventricular myocardium, even at nanomolar concentrations, PTHrP was able to activate adenylate cyclase, exerting a positive inotropic and isotropic effect in adult ventricular cardiomyocytes and improving cardiac function with a greater potency than PTH. In addition, PTHrP action increased protein mass and p42-MAP kinase activity in the myocardium while it induced re-expression of certain fetal-type proteins, such as creatine kinase BB in cardiomyocytes, *in vitro*. Notably, PTHrP activated protein kinase C in cardiomyocytes, leading to the acceleration of protein synthesis *via* an as yet unknown mechanism (54). Moreover, activation of the PTH-1.R receptor leads to an increase in the intracellular calcium that can be abolished by the calcium channel blocker verapamil (54, 55). Subsequently, increased calcium levels activate protein kinase C, activating hypertrophic processes (56). In addition, the importance of the PTHrP/PTH-1.R bioregulation system in cardiovascular development was indicated by the fact that deletion of either PTHrP or PTH-1.R resulted in a higher incidence of early fetal death at days 9-10, which coincide with the development of the heart and major blood vessels (57).

In a clinical setting, the serum PTHrP concentration was shown to correlate positively with the degree of cardiac dysfunction of patients with chronic heart failure (58, 59), suggesting that PTHrP participates in the pathophysiology of cardiac hypertrophy/heart failure. Apparently, the co-expression of the calcium (PTHrP-dependent) and sodium (ANP-dependent) regulation systems in the myocardium suggests that these two ion-regulating systems may participate in crucial pathophysiological processes in human myocardium.

Both pressure-overload and volume-overload induced hypertrophy are characterized by distinct cellular phenotypes (7, 8). This heterogeneity at the molecular level presumably reflects differences in the type of hemodynamic overload-activated signaling pathways. Indeed, coronary endothelial cells were shown to enhance PTHrP mRNA

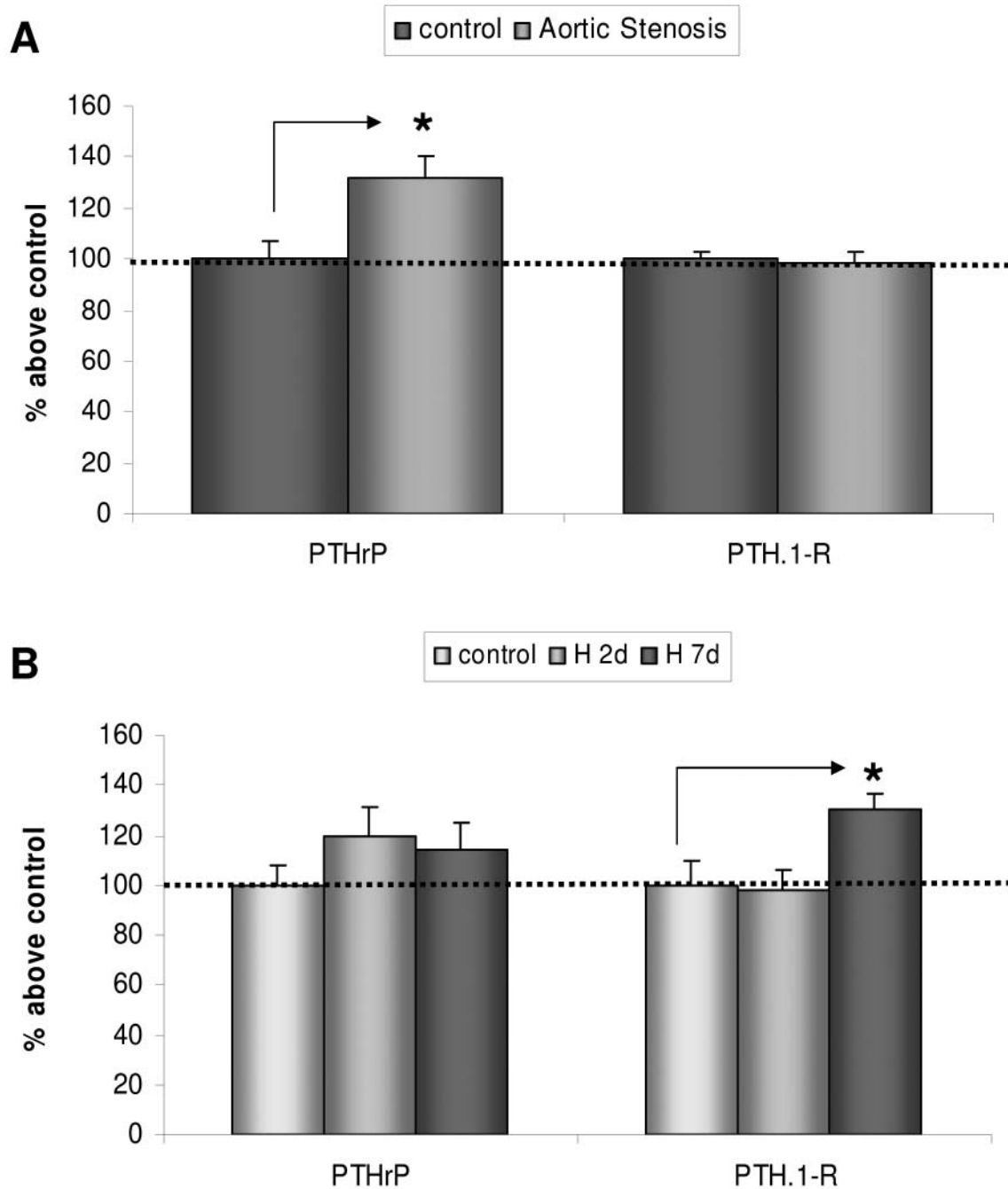


Figure 2. Parathyroid hormone related protein (PTHrP) mRNA expression is increased after surgically-induced aortic stenosis (Panel A), while type 1 PTH receptor (PTH.1-R) mRNA is overexpressed during hyperthyroidism induced hypertrophy (Panel B) in rat ventricular myocardium. Notably, the acute hyperthyroidism state (H-2d) did not significantly influence the relative PTHrP and PTH.1-R mRNA expression in rat ventricular myocardium, suggesting that thyroid receptor (TR) trans-activation does not directly alter PTHrP and PTH.1-R gene expression, in rat myocardium. (* $p<0.05$).

expression during aortic constricted overload-induced hypertrophy while cardiomyocytes became more sensitive to PTHrP action by overexpressing PTH.1-R in hyperthyroidism-induced hypertrophy of rat ventricular

myocardium (Figure 2) (60). Whether this represents a similar pattern of PTH/PTH.1-R expression in the volume overload and pressure overload model requires further investigation. Consequently, there is possibly a cause related

increase of the transcription of either the PTHrP or PTH-1.R gene in the development of rat ventricular hypertrophy.

It is well known that PTH acts *via* the PTH-1.R receptor. The fact that PTH-1.R participates in the development of cardiac hypertrophy derives from *in vivo* studies in patients with hyperparathyroidism (HPT). Indeed, in HPT there is an increased prevalence of ventricular hypertrophy which seems to be independent of blood pressure (61, 62). This is in line with observations that cardiac hypertrophy is a common finding in normotensive HPT patients (62). In patients with secondary hyperparathyroidism, there is an increased prevalence of left ventricular hypertrophy, which was reversed after parathyroidectomy and a subsequent reduction of PTH levels (63). This may further strengthen the notion that the PTH-1.R participates in the hypertrophic mechanism.

This corroborates the data of previous studies, which have provided strong evidence that the PTHrP/PTH-1.R bioregulation system is possibly part of the pathophysiological processes in the myocardium. These studies have documented that the expression of PTHrP in vascular smooth muscle cells (VSMC) is regulated by vasoconstrictors, such as norepinephrin, endothelin-1, angiotensin-II, serotonin, bradykinin and thrombin, and by mechanical stress, such as mechanical distention of the vascular wall (64-66). Other experimental data have shown that PTHrP is up-regulated after balloon angioplasty and by increasing arterial blood pressure (67). In addition, mechanical stress induces the expression of PTHrP in VSMC while alterations in blood flow promote a mechanosensitive release of PTHrP from coronary endothelial cells (68). Interestingly, TNF- α and Interleukin-1 β enhanced PTHrP production in a time- and dose-dependent manner, *in vitro* (69). Moreover, several *in vitro* studies have reported that TGF β -1 down-regulates PTHrP production in vascular endothelial cells from rat ventricular myocardium (70). However this has not been confirmed in other cell types, thus reinforcing the concept that PTHrP may be implicated among other local growth factors in myocardial hypertrophy.

Conclusion

Cardiac hypertrophy develops in response to an increased hemodynamic overload and represents a significant risk factor for cardiovascular morbidity and mortality. The precise mechanism through which an excessive hemodynamic load promotes cardiac hypertrophy is not fully understood, however, it could include the PTHrP/PTH-1.R bioregulation system. The elucidation of the precise role of the PTHrP/PTH-1.R bioregulatory system in cardiomyocytes and in vascular smooth muscle cells, as well as the characterization of specific substances/modulators of the PTHrP/PTH-1.R signal transduction activity may open new avenues for the treatment of cardiac hypertrophy and cardiac failure.

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