Open Access

Role of Extracellular Calcium Control, Calcium Sensing, and Regulation of Calcium Regulating Hormones in Heart Failure

R. Schreckenberg, S. Wenzel and K.-D. Schlüter*

Justus-Liebig-Universität Giessen, Germany, Physiologisches Institut, Aulweg 129, 35392 Giessen, Germany

Abstract: Calcium plays a pivotal role in excitation-contraction coupling of cardiomyocytes and many other cellular responses observed in cardiovascular cells. Thus maintaining a healthy status requires very strict regulation of cytoplasmatic but also plasma ionized calcium concentration. Plasma ionized calcium is regulated by calcium sensing and the regulation of calcium uptake and secretion. Under conditions of heart failure, however, electrolyte deregulation occurs due to an activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system because both systems are coupled calcium regulation and thereby also to the regulation of hormones controlling calcium homeostasis such as parathyroid hormone, parathyroid hormone-related protein, and vitamin D that are activated in a calcium-dependent way. Of note, these hormones and receptors have also direct cardiac effects that modulate cardiac and renal function. Therefore, they play not only a role in end-stage heart failure but also in essential hypertension and reno-cardiovascular complications. In this review we summarize our current understanding about the role of calcium deregulation in heart failure and discuss the consequences from these observations. In conclusion, controlling plasma ionized calcium, plasma parathyroid hormone, and vitamin D status are pivotal in successful pharmacotreatment of patients with heart failure.

Keywords: Parathyroid hormone, PTH receptors, calcium sensing receptor, vitamin D, aldosterone, kidney.

1. CALCIUM CONTROL IN HEART FAILURE

Systemic calcium control is crucial for the maintenance of a normal and healthy status. Therefore, plasma calcium levels are strongly controlled by various mechanisms including the control of calcium uptake, calcium release, and calcium mobilization from the bone. In these processes hormones like parathyroid hormone (PTH) and vitamin D are involved. As a result of these control mechanisms plasma calcium concentrations are strictly controlled and show little variations. However, the above mentioned control mechanisms are embedded in a complex network of regulator circuits that play also a role in cardiovascular regulation. The most prominent example is that of the renin-angiontensin-aldosteron system (RAAS). The RAAS is activated as a compensatory mechanism that counterbalances reduced pump activity of the heart and it is one key pathway that is deregulated in heart failure. As the release of renin is regulated by the sympathetic nervous system, RAAS is also connected to sympathetic hyperactivation, another key event in heart failure. As a consequence, renin is released from the kidney and promotes the formation of angiotensin I from angiotensinogen. Angiotensin I is subsequently converted to angiotensin II (Ang II) *via* the angiotensin-converting-enzyme (ACE). The formation of Ang II by ACEs is a complex system, including different isoforms of ACE, different peptidases, and finally different angiotensin I-derived peptides that act on various receptor subtypes again. This will not be discussed in this review but has recently been summarized [1]. In the context of calcium regulation, Ang II seems to be the most important peptide among the different angiotensin I-derived peptides. It induces the release of aldosterone from the adrenal cortex and therefore it indirectly favours urinary and faecal calcium extrusion. Thus, patients with heart failure are at an increased risk of hypocalcaemia. This fall of calcium levels is detected by the calcium(-sensing) receptor (CaSR) of the parathyroidea that results in an increased release of PTH (Fig. **1**). But what is the evident that patients with heart failure develop indeed hypocalcaemia? In blood samples from patients with chronic heart failure a linear correlation exists between the concentration of ionized calcium and the NYHA classification of the patients [2]. This indicates that hypocalcaemia is indeed present in severely ill patients with chronic heart failure (Fig. **2**). Ionized calcium directly modifies pacemaker activity in the heart and it is a key player in excitation-contraction coupling in cardiomyocytes [3-5]. We could recently show that reducing extracellular calcium to values depicted in the plasma of patients with chronic heart failure in the NYHA classification IV directly reduced the contractile performance of cardiomyocytes underlining the relevance of small changes in extracellular calcium for heart function [6]. Lower ionized calcium levels in patients with heart failure seem to be a common finding. In African-Amercian patients hospitalized with chronic heart failure lower ionized calcium levels were measured [7]. Noteworthy, Carlstedt *et al*. [8] found a significant difference in ionized calcium between patients in the emergency department that survived and those that did not survive. Patients that did not survive had again lower ionized plasma levels. Although hypocalcaemia is not restricted to patients with heart failure in an emergency department, patients with acute myocardial infarct and heart failure represented the most remarkable subgroup. Again, severity of illness and survival were related to less ionized calcium. Of

*Address correspondence to this author at the Justus-Liebig-Universität Giessen, Germany, Physiologisches Institut, Aulweg 129, 35392 Giessen, Germany; Tel: +49 641 99 47 212; Fax: +49 641 99 47 219;
E-mail: Klaus-Dieter.Schlueter@physiologie.med.uni-giessen.de

1876-5351/10 2010 Bentham Open.

note is the observation that the use of loop diuretics further improves the excretion of calcium and thereby will intensify the problem. The importance of lost minerals in heart failure has recently been reviewed [9].

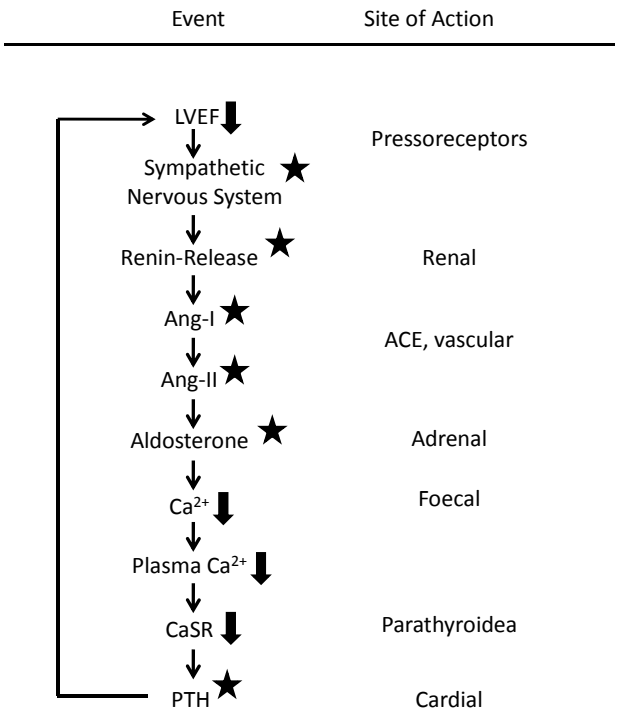

Fig. (1). Event sequence and localization of events involved in neurohumoral changes that influence systemic calcium handling. LVEF = left ventricular ejection fraction; *, indicates activation or induction; arrows indicate release or fall of the corresponding parameter.

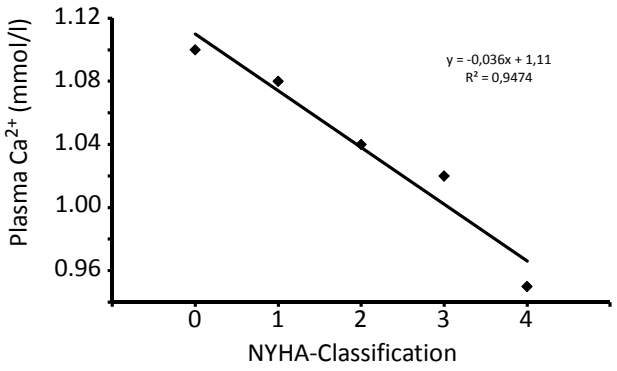

Fig. (2). Association between plasma ionized calcium and NYHA classification in patients with heart disease (data depicted from Aralkelyan *et al*., 2007).

Similar to the above mentioned relationship between NYHA classification and loss of ionized plasma calcium, plasma ionized calcium levels also fall in aldosterone treated rats [10]. These rats seem to represent a reasonable model to study the effect of plasma calcium deregulation on heart failure. The data on aldosterone treated rats suggest that the loss of ionized plasma calcium in heart failure is indeed initiated by the alterations of the RAAS. However, direct evidence that plasma ionized calcium levels will be normalized again by the administration of aldosterone antagonists in patients with heart failure is still lacking. Unfortunately, many studies dealing with the relationship between calcium homeostasis and heart failure investigated total calcium but not ionized calcium levels and cannot address this question.

In summary, there is compelling evidence that reduced ionized calcium is common in patients with heart failure resulting in compensatory elevation of PTH. This has strong implications in cardiac function due to the interaction of calcium with the CaSR expressed in the parathyroidea but also in cardiomyocytes in which the CaSR contributes to calcium load of cardiomyocytes [11].

2. PTH LEVELS IN HYPERTENSION AND HEART FAILURE

Following the assumptions outlined in the previous chapter one must predict that patients with heart failure and lower levels of plasma ionized calcium develop an increased release of PTH from the parathyroidea. There are many studies that have addressed the question whether heart failure is associated with increased plasma PTH in clinical settings. In example, in the above mentioned study [7] serum PTH was elevated in all patients with chronic heart failure of four weeks or longer duration. In a small number of patients with chronic left ventricular dysfunction (EF <35%) PTH levels were above the normal range [12]. Surprisingly, patients with a combined treatment consistent of ACE inhibitors, loop diuretics (furosemide) and small-dose aldosterone antagonist spironolacton still had PTH levels above the normal range. Arakelyan *et al*. [2] reported about a correlation between plasma PTH and NYHA classification. Elevated PTH was also found in patients with end stage heart failure awaiting cardiac transplantation [13]. Of note, in heart failure patients PTH levels are not only elevated under basal conditions but PTH release under stress conditions is also depressed [14]. As PTH release is linked to the CaSR, a receptor directly contributing to cardiac performance, one must predict a defect in calcium handling in myocytes as well. The increase in PTH may be caused by aldosterone-dependent sodium retention, use of loop diuretics in such patients, or hypovitaminosis D as outlined later and reviewed in more detail before [15]. A very recently published analysis based on nearly 1,000 patients revealed that plasma PTH predicts cardiovascular mortality, even in individuals with PTH within the normal range [16]. Noteworthy, this holds after adjustment for established cardiovascular risk factors, indicating that PTH plays an independent role in cardiovascular disease.

Preliminary, PTH seems to play a major role in hypertension. A fall in plasma ionized calcium levels leads to more PTH release from the parathyroidea. In spontaneously hypertensive rats, the best investigated hypertensive model in respect to calcium deregulation, hypertension can be reduced by high-calcium diet or parathyroidectomy [17-19]. Moreover, administration of PTH is sufficient to reverse the effect of high calcium diet or parathyroidectomy [20]. Even administration of calcimimetics, acting on CaSRs of the parathyroidea, seems to attenuate high blood pressure *via* decreased PTH release [21]. It is nevertheless questionable whether dietary calcium uptake is sufficient to improve patients' outcome. In spontaneously hypertensive rats high

calcium diet normalized plasma ionized calcium levels and PTH levels, but did not alter the progression of hypertensive heart disease [22] or even did not normalize plasma ionized calcium levels [23]. High calcium intake may stimulate renin production in the kidney which in turn raises Ang II levels, another factor leading to heart failure [24-26]. In contrast to the association between PTH and hypertension, PTH acts as a vasodilator even in chronic hypertensive vessels [27]. It is well known that the expression of PTH receptors is regulated in a tissue-specific way. In spontaneously hypertensive rats, angiotensin II destabilizes PTH receptor transcripts and thereby down-regulates renal PTH receptors independent of the blood pressure in the kidney [28]. *Via* this mechanism, kidney perfusion is reduced and renin release increases as well as that of other renal hormones. Similarly, PTH receptor expression is dramatically decreased in folic-acid induced acute renal failure in rats [29]. However, in twenty patients with proven primary hyperparathyroidism only a weak correlation between PTH and plasma renin activity was found [30]. On the other hand, patients with primary hyperparathyroidism display higher plasma renin activity at least in those subgroups that developed hypertension [31]. It has been assumed that the effect on renin release overrides the potential blood pressure lowering effect of PTH observed in peripheral vessels.

In summary, the data on spontaneously hypertensive rats and on patients with primary hyperparathyroidism suggest a possible relationship between PTH, kidney-derived down-regulated PTH receptors, and increased plasma renin activity. Another mechanism by which PTH acts on kidneys and influences electrolyte balance and thereby indirectly blood pressure and favours heart failure is by induction of glomerular hyperfiltration. Down-regulation of PTH receptors in the kidney will produce vasoconstriction.

3. DIRECT CARDIAC EFFECTS OF PTH

Cardiovascular effects of PTH have been recognized as early as the early 80ths of the last century [32]. Since that the role of excessive PTH on cardiac cells under conditions such as secondary hyperparathyroidism has been addressed. In general it has been accepted from such studies that excessive PTH has a direct deleterious effect on cardiomyocytes. As cardiovascular complications are the main secondary effect of secondary hyperparathyroidism it seems reasonable to assume that PTH acts directly on cardiomyocytes thereby influencing hypertrophic growth control, contractile responsiveness, and energy metabolism [33-35]. As a main finding of these studies, PTH was identified as a factor that increases calcium load and activates phospolipase C-dependent pathways. However, an unsolved problem was still to explain why these cells should express a PTH receptor. The identification of sister-peptides such as parathyroid hormone-related peptide (PTHrP), and moreover the observation that PTHrP is synthesised and released from microvascular coronary cells in the heart, suggests that PTHrP acts in a paracrine matter on cardiomyocytes. The observed cardiac effects of PTH on cardiomyocytes are then attributed to the structural homology between PTH and PTHrP allowing both peptides to act on the same receptor. This explains the responsiveness of cardiomyocytes to PTH. However, a correlation between PTH and cardiovascular events as described above requires PTH effects at concentrations much below the levels found in patients with secondary hyperparathyroidism. Therefore, the question about the role for PTH in heart failure in patients without secondary hyperparathyroidism was still obscure. Only recently two distinct ways of receptor-ligand interaction were described for PTH on cardiomyocytes. PTH displays two functional domains in its N-terminal part that is represented by the amino acids 1-34. *Via* the first six amino acids PTH binds to a Gαs-coupled PTH receptor of cardiomyocytes and activates adenylyl cyclase. In principle this should result in an increase of calcium influx that promotes cardiac function. However, at physiological or high-physiological extracellular calcium concentrations extracellular ionized calcium does not only move into the cell but also binds to cardiac CaSRs and activates them. CaSRs have been shown to adversely affect the activation of PTH receptors [36]. Therefore, as higher the extracellular calcium concentration is as less effective are PTH-dependent effects on cell shortening. A drop in ionized calcium results in less calcium influx and less activation of CaSRs. Therefore, PTH can now compensate for the loss of calcium influx by lower calcium concentrations (Fig. **3**). Moreover, PTH down-regulates CaSRs and therefore further potentiates its effect on cardiac performance at low extracellular ionized calcium [6]. As a consequence, PTH compensates for the lower plasma ionized calcium levels. Noteworthy, this occurs at very low concentrations of PTH that are not sufficient to improve cell function in a direct way comparable to other Gαs-activating agonists such as norepinephrine [37].

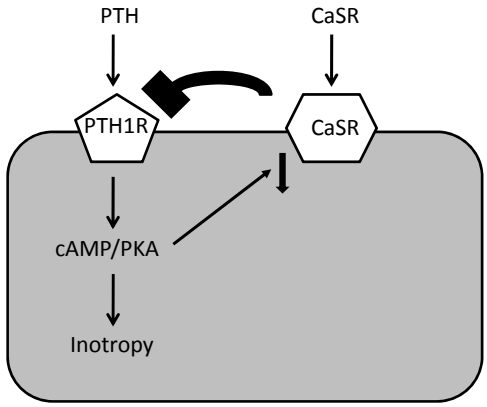

Fig. (3). Interaction of PTH receptors and CaSRs on cardiomyocytes. Normal plasma calcium leads to a CaSR-dependent inhibition of PTH signalling, while activation of PTH receptors will down-regulate CaSR and thereby intensify its action on inotropy.

PTH displays another functional domain in its first 34 amino acids, namely that covered by amino acids 28-32. *Via* these amino acids PTH activates Gαq-coupled PTH receptors that trigger activation of phospholipase C and subsequently that of protein kinase C [38]. This activation requires higher concentrations of PTH than that responsible for the effects on cell shortening *via* cAMP/PKA. It is linked to induction of protein synthesis thereby inducing cardiac hypertrophy. It is most likely that this mechanism contributes to cardiac side effects seen in patients with primary hyperparathyroidism which is associated with increased prevalence of left ventricular hypertrophy independent of blood pressure [39]. PTH also plays an important role in secondary hyperparathyroidism. This is a clinical relevant problem as patients with

heart failure and renal failure display a poor prognosis compared to patients with heart failure alone (reviewed by [40]). Renal failure requiring hemodialysis is associated with an increased risk of secondary hyperpararthroidism. The severity of secondary hyperparathyroidism in chronic renal insufficiency is associated with cardiovascular disease. PTH was associated with myocardial infarction and congestive heart failure in these patients [41]. A case report on 52-year-old women with secondary hyperparathyroidism and left ventricular dysfunction showed a marked improvement of left ventricular function after parathyroidectomy indicating the contribution of PTH in left ventricular dysfunction [42]. Similarly, in a cohort study on patients with primary hyperparathyroidism patients who underwent surgery had a lower prevalence of acute myocardial infarction and stroke and mortality was lower [43]. On the other hand, regression of left ventricular hypertrophy in patients on high-flux haemodialysis did not occur when PTH levels remained above 300 pg/ml [44]. All these data argue for a main role of PTH in left ventricular hypertrophy and dysfunction in patients with hyperparathyroidism.

But how can we explain the difference between cAMP/PKA- and PKC-dependent effects in a mechanistic way? PTH-receptors are G-protein coupled receptors belonging to the same receptor family as calcitonin gene related peptides. This receptor superfamily is characterized by an interaction between receptors and so-called receptor associated modifying proteins (RAMPs). Calcitonin gene-related peptide (CGRP) receptors are prominent members of this superfamily. In those receptors, RAMPs decide about the affinity to CGRP, adrenomedullin, and intermedin (=adrenomedullin 2). It may be that the coupling of PTH receptors to either Gαs or Gαq depends on the interaction between PTH receptor molecules and different RAMPs. For CGRP it has been shown that this results in a concentration-dependent increase or decrease of cell shortening on cardiomyocytes [45]. Of note, expression of RAMP isoforms is changed in rats with chronic heart failure thereby changing ligand-dependent receptor activation under these conditions [46].

Stimulation of PTH receptors results in a different inotropic responsiveness in adult versus young rats [47]. Interestingly, in most of the aforementioned studies patients were rather old and in these patients a close relationship between PTH and heart failure was found. One may speculate that PTH compensates for variations in ionized calcium concentrations in young individuals but that the same mechanism contributes to heart failure in elder individuals. It may also be that this different behaviour depends on alterations in RAMP proteins. However, this hypothesis requires future studies to be verified and is a pure speculation drawn from present studies at the moment.

The regulation of PTH receptors in cardiomyocytes has not been analyzed so far in deep. Given the known role of PTH and PTH signalling in heart failure it is of emerging interest to understand the regulation of receptor expression and coupling. Most recently, it was found that the regulation of cardiac rather than vascular PTH receptors is of particular relevance in postmenopausal females. Nitric oxide (NO) deficiency leads to a down-regulation of PTH receptors [48]. Oestrogen contributes significantly to the local generation of NO. Consequently, ovariectomized rats had a nearly 40% lower left ventricular steady state mRNA level of PTH receptors and this deficit could be nearly normalized by oestrogen supplementation. Keeping in mind that PTH receptors act different in hearts from old or young rats these data can be seen again as a compensatory down-regulation of PTH receptors in elder individuals. Noteworthy, hormone-replacement-therapy in post-menopausal women has unexpectedly been combined with increased cardiovascular events although oestrogen should be cardioprotective [49]. A possible explanation may be that down-regulation of PTH receptors in elder hearts is a compensatory mechanism and that replenished oestrogen pools increase its expression again.

4. INDIRECT CARDIAC EFFECTS OF PTH

Despite the effect of PTH on cardiomyocytes that has been addressed above, non-direct effects of PTH on cardiac performance must also be taken into consideration due to the strong correlation between PTH and heart failure. PTH acts on its classical targets such as kidney and bone. Special interest has been drawn to the effect of PTH on bone turnover with respect to cardiovascular function. PTH mobilizes the release of stem cells from the bone niche. In an infarct model, cardiac expression of SDF-1 is increased and this recruits more stem cells to the heart [50] (Zaruba *et al.*, 2008). In the presence of PTH in which more stem cells are mobilized from the bone niche, stem cell recruitment is optimized by a more intensive release of such cells from the bone marrow and a direction of such cells to the heart by SDF-1 (reviewed by [51]). It is still an open question at the moment to what extent such cells contribute to post-infarct remodelling and by which mechanisms. However, there is compelling evidence that PTH directly improves stem cell mobilization. Whether the moderate high PTH plasma levels in patients with heart failure are already sufficient to increase stem cell mobilization is nevertheless unclear.

Finally, PTH has vasodilatory properties that are independent of its effect on cardiomyocytes. PTH hyperpolarizes vessels and therefore reduces vascular resistances [52]. Whether a cAMP-dependent or NO-dependent effect contributes to its vasodilatory effect is not completely understood at present. By hyperpolarization of endothelial cells PTH can act even on cells with endothelial dysfunction due to a reduction of endothelial NO synthase activity. Although all experimental data on direct effects of PTH on peripheral resistances indicate a vasorelaxant effect of PTH the hormone is associated with high blood pressure. It is still unclear why PTH acting as a vasodilatory hormone contributes to high blood pressure. From the clinical point of view, however, it is important to note that PTH cannot compensate for the induction of high blood pressure under chronic conditions. Thus it remains unclear at the moment to what extent and under which conditions PTH modifies the peripheral resistances.

5. PTHrP IN HEART FAILURE

The identification of the formerly named hypercalcemia factor of malignancy as PTHrP and the discovery of a high structural similarity to PTH has greatly improved our understanding why cardiovascular cells express a receptor that responds to PTH and PTHrP. Cardiac PTHrP is synthesized

and released by endothelial cells [53]. This suggests that PTHrP acts as a paracrine factor in cardiac physiology. Indeed, the role of locally produced PTHrP in the vascular system has been established in transgenic mice that constitutively overexpress PTH receptors in smooth muscle cells and develop lower blood pressure [54, 55]. Furthermore, a decrease in blood pressure has been observed in adult rats with overexpression of PTH receptors in the vessels after i.v. delivery of PTH receptor cDNA [56]. It was found that PTHrP is locally up-regulated in a pressure-dependent manner and released by mechanical stress consistent with the role as a negative feedback mechanism to oppose the myogenic contractile response [57]. There are less studies dealing with plasma PTHrP levels and heart failure than those analyzing plasma PTH and heart failure. A direct comparison gave a strong increase of PTH and PTHrP in correlation to NYHA classification [2]. This can be explained in part related to a pressure-dependent release of PTHrP from endothelial cells. Of note, plasma PTHrP in females exceeded the values depicted in males. As one may assume from this observation, PTHrP expression is regulated at least in part in an oestrogen-dependent way [58]. Vascular PTHrP expression is down-regulated by TGF-β1 but left ventricular expression of PTH receptors seems to be up-regulated [59-61]. Therefore, in heart failure PTHrP is predominantly down-regulated by an increased expression of corresponding PTH receptors that is just the opposite of what is known from the kidney (Fig. **4**). Noteworthy, in contrast to the study mentioned above, in which an association between plasma PTHrP and NYHA classification was found, an inverse correlation between plasma PTHrP and NYHA classification was found as well [62]. The latter represents more closely the down-regulation of microvascular PTHrP in the heart although completely opposite results in two different studies do not allow any strong judgement at the moment. A problem with the different studies may be that PTHrP undergoes a strong posttranslational modification. Therefore, antibody-driven analysis might display different isoforms of PTHrP that lead to different results. It is nevertheless quite clear that increased myocardial PTHrP expression leads to a local release of PTHrP from the myocardium [57, 63]. Interestingly, TGF-β1 is induced in pressure-overloaded hearts at the transition from adaptive to mal-adaptive hypertrophy. Therefore in the chronic hypertensive heart a decrease in PTHrP expression is expected to be accompanied by an increased cardiac expression of PTH receptors and an increase in plasma PTH. This shifts the contribution of locally delivered PTHrP to PTH. If these alterations have any prediction in the scenario leading to heart failure than PTH and PTHrP must exert different effects on cardiac tissues. Indeed, despite a structural similarity between both peptides a detailed analysis of the structure-function relationship reveals strong differences.

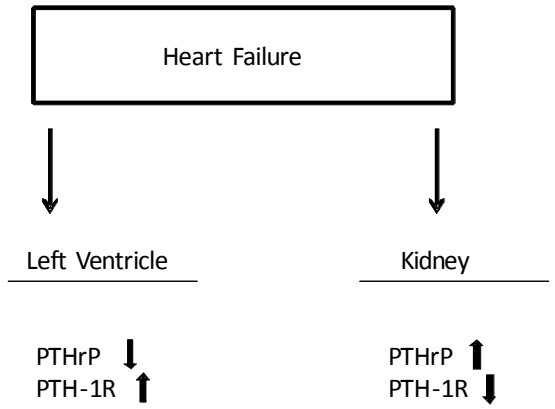

Fig. (4). Differential regulation of PTHrP and the corresponding receptor in the left ventricle and the kidney in patients with heart failure.

PTHrP also affects blood pressure control *via* renal mechanisms. In the kidney any increase in blood pressure increases PTHrP expression. However, renal PTHrP-dependent vasodilation is blunted in spontaneously hypertensive rats due to a down-regulation of the corresponding receptors. Furthermore, PTHrP is a potent stimulator of renin release by direct interaction with juxtaglomerular cells [64]. Modulation of renal PTHrP receptor expression in these rats increased renovascular responsiveness to PTHrP but did not modify blood pressure due to a corresponding 60% increase in plasma renin activity [65].

6. CARDIOVASCULAR EFFECTS OF PTHrP

As mentioned above PTHrP and PTH share structural similarities in their N-terminal part leading to a similar binding to a common receptor and activation of similar pathways. However, PTHrP is a peptide formed in three different lengths in humans (1-139, 1-141, and 1-173) and is target of proteolytic cleavage and glycosylation. This high amount of post-translational modification leads to the formation of various locally produced peptide variants. Therefore, PTHrP and PTH display significant different physiological effects. In respect to their interaction with cardiomyocytes this means they have a different impact on inotropy and hypertrophic growth regulation. While PTH improves contractile performance of cardiomyocytes preferentially *via* its N-terminal part (amino acids 1-6) at picomolar concentrations in a non-acute way, PTHrP improves cardiomyocytes performance *via* a cAMP/PKA-dependent pathway involving amino acids 6-11 in a more acute way at higher concentrations [6, 66]. Further, PTH exerts a positive hypertrophic effect *via* its PKC-activating domain covering amino acids 28-32, while a PKC-activating domain of PTHrP is located at amino acid 107-111 and therefore be represented by a C-terminal degradation peptide, known as osteostatin [66]. It is still an open field to identify those *in vitro* effects that are the most relevant *in vivo*. In terms of acute regulation of inotropy, cardiomyocytes seem to be more sensitive to PTHrP than to PTH. However, long-term effects might be more effectively evoked by PTH than by PTHrP.

In respect to vascular effects of either PTH or PTHrP, PTHrP differs from PTH because it exerts paracrine and intracrine effects whereas PTH acts as an endocrine factor. The intracrine effect of PTHrP depends on an active nuclear location site (NLS) represented by amino acids 87-107. Therefore, PTHrP unlike PTH can influence smooth muscle cell proliferation *via* receptor-dependent and receptor-independent effects. Interestingly, the effect of PTHrP on smooth muscle cell proliferation evoked by receptor-dependent processes is opposed by its intracrine effects [67]. Therefore, PTHrP plays a complex role in the progression of atherosclerotic plaques, yet not fully understood. Similarly, PTHrP acts on endothelial cells which represent the main

source of the peptide in the heart [52, 60, 68]. There is some evidence that PTHrP can enter the nucleus of endothelial cells as well, however, a precise role for this intracrine activity on endothelial growth and apoptotic signalling has not been described. Further, PTH unlike PTHrP can bind to and activate at least two different receptor subtypes (PTH-1 receptor and PTH-2 receptor) that influence agonist-dependent signalling in a contradictory matter [69]. Therefore, the question whether PTHrP controls basal blood pressure is at least dependent on the ratio between expressions of both receptor subtypes.

Finally, it should be mentioned that another member of this peptide family acts on PTH receptor subtypes, namely tuberoinfundibular peptide of 39 amino acids (TIP39). The latter one is known to influence inotropy of the heart *via* PTH-2 receptors and seems to be the physiological agonist of this receptor rather than PTH [70]. Whether this represents a redundant system to maintain normal pump function or whether it plays a genuine role in heart failure is not clear at present and requires additional research.

7. VITAMIN D, SYSTEMIC CALCIUM REGULATION, AND HEART FAILURE

The aforementioned increase in plasma PTH is initiated by a fall in plasma ionized calcium. Reduced levels of vitamin D, specifically of the most active vitamin D metabolite, 1,25-dihydroxyvitamin D3 or calcitriol, are common in patients with heart failure [71-73]. It is reasonable to assume that low vitamin D status contributes to low levels of plasma ionized calcium. In spontaneously hypertensive rats, vitamin D3 normalized plasma ionized calcium levels [74]. Furthermore, as vitamin D3 is a negative regulator of renin production its lower availability is associated with an activation of the RAAS [24]. However, vitamin D-receptor ablation in knockout mice (VDRKO mice) was not accompanied by elevated plasma renin levels, possibly because mice and rats display a different regulation of renin formation [75]. Alternatively, vitamin D3 may exert cardiac effects *via* an inhibition of leptin synthesis. The latter observation might be of cardiovascular relevance because cardiovascular cells express leptin receptors. Noteworthy, vitamin D treatment reduced cardiac hypertrophy in SHHF (cp/+) rats [76]. It has been proposed that low vitamin D status is caused by lower outdoor activity of patients with heart failure [77]. This hypothesis is supported by studies such as those that observed seasonality in heart failure deaths and total cardiovascular deaths in Australia [78]. However, vitamin D is added to many nutrients and it is questionable whether low outdoor activity is indeed responsible for low vitamin D levels in patients with heart failure. As mentioned above, renal expression of PTH receptors is lower in chronic hypertensive patients. Therefore, PTH-mediated renal formation of 1,25-dihydroxyvitamin D3 might also be impaired. Alternatively, a genetic abnormality of the hepatic 25-hydroxylase activity has been described as a reason for hypovitaminosis D [79]. Nevertheless, lower vitamin D impairs calcium uptake and contributes to the fall in plasma ionized calcium. Therefore, low vitamin D is associated with already mentioned effects of lower calcium and higher PTH on cardiovascular cells. Despite these effects, vitamin D deficiency is associated with altered cardiac function and hypertension in rat models that are independent of any effects on calcium homeostasis [80]. Furthermore, cardiac myocytes express functionally coupled vitamin D receptors [81]. Vitamin D affects directly contractile performance of cardiomyocytes in a PKC-dependent mechanism that leads to a phosphorylation of phospholamban and troponin I [82]. Although a direct comparison between effects of vitamin D on cardiovascular cells and effects linked to lower calcium uptake has not been worked out, it is reasonable to assume that vitamin D modifies cardiovascular physiology at different levels. Based on the aforementioned physiological activities of vitamin D3 it has been suggested that vitamin D deficiency is an underestimated non-classical risk factor for cardiovascular disease, specifically in patients with chronic kidney disease [83]. There is also some clinical evidence that vitamin D and calcium diet decreases inflammatory markers in patients with heart failure although a subsequent follow-up of this study did not further indicate an additive effect of vitamin D on calcium diet [84, 85].

8. ALDOSTERONE AND SYSTEMIC CALCIUM REGULATION IN HEART FAILURE

Aldosterone is part of the endocrine deregulation that is associated with left ventricular dysfunction because it is linked to the renin-angiotensin system. Classical endocrine effects of aldosterone are focussed on the kidney where the mineralocorticoid hormone aldosterone improves sodium retention and thereby volume regulation. The additional effect on calcium and magnesium secretion has been mentioned before [10]. Of note, loop diuretics exaggerate the losses of calcium and magnesium evoked by aldosterone and therefore increase electrolyte deregulation independent of direct cardiovascular effects evoked by aldosterone [86]. Aldosteronism is coupled to secondary hyperparathyroidism as the loss of ionized calcium increases PTH release, that favours cell uptake of calcium associated with altered function of target cells such as cardiomyocytes [34] and peripheral blood mononuclear cells [87]. As a consequence aldosteronism leads to cardiac oxi/nitrosative stress and a proinflammatory phenotype [15]. Despite this, aldosterone has direct effects on cardiomyocytes. In an acute way aldosterone modifies contractile responsiveness but also as a long-term effect [88]. *In vivo* the endocrine and cardiovascular effects lead to an improvement of fibrosis in hearts adding another risk factor to the long-term prognosis. In the light of these experimental data, aldosterone antagonism has been introduced into clinical practice and is now part of advanced pharmacotherapy in patients with heart failure. Of note, the concentration of aldosterone used during *in vitro* studies is far above the levels found *in vivo*. However, conclusions drawn from such experiments have lead to the introduction of aldosterone antagonism in clinical practice and lead to a significant reduction in total mortality and mortality linked to cardiovascular events. Therefore, the non-physiological high concentration of aldosterone used in these *in vitro* studies is properly linked to the experimental system but does not exclude these studies from (patho)physiological relevance per se.

CONCLUSIVE REMARKS

Calcium deregulation is commonly found in patients with heart failure. This is associated with deregulation of calcium homeostatic controlling hormones, such as PTH and vitamin

D, a deregulation of the corresponding receptor expression and signalling, and direct and indirect effects of such hormones on cardiovascular cells. Although deregulation can be observed at different levels, PTH and plasma ionized calcium concentration seem to have an outstanding role specifically in the elderly. As these mechanisms are linked to classical neurohumoral regulatory circuits that control cardiovascular physiology, a better understanding of the molecular and cellular effects under physiological and pathophysiological conditions seems to be a key to improve our current pharmacotherapy of patients with heart failure that have still a poor prognosis.

REFERENCES

[1] Schlüter KD, Wenzel S. Angiotensin II: A hormone involved in and contributing to pro-hypertrophic networks and target of anti-hypertrophic cross-talks. Pharmacol Ther 2008; 119: 311-25.

[2] Aralkelyan KP, Sahakyan YA, Hayrapetyan LR, *et al.* Calcium-regulating peptide hormones and blood electrolyte balance in chronic heart failure. Regul Pept 2007; 142: 95-100.

[3] Cleemann L, Morad M. Role of Ca^{2+} channel in cardiac excitation-contraction coupling in the rat: evidence from Ca^{2+} transients and contraction. J Physiol 1991; 432: 283-312.

[4] Levi AJ, Brooksby P, Hancox JC. One hump or two? The triggering of calcium release from the sarcoplasmatic reticulum and the voltage dependence of contraction in mammalian cardiac muscle. Cardiovasc Res 1993; 27: 1743-57.

[5] Wang SQ, Song LS, Lakatta EG, Cheng H. Ca^{2+} signalling between single L-type Ca^{2+} channels and ryanodine receptors in heart cells. Nature 2001; 410: 592-6.

[6] Tastan I, Schreckenberg R, Mufti S, Abdallah Y, Piper HM, Schlüter KD. Parathyroid hormone improves contractile performance of adult rat ventricular cardiomyocytes at low concentrations in a non-acute way. Cardiovasc Res 2009; 82: 77-83.

[7] Laguardia SP, Dockery BK, Bhattacharya SK, Nelson MD, Carbone LD, Weber KT. Secondary hyperparathyroidism and hypovitaminosis D in African-Americans with decompensated heart failure. Am J Med Sci 2006; 332: 112-8.

[8] Carlstedt F, Lind L, Wide L, *et al.* Serum levels of parathyroid hormone are related to the mortality and sensitivity of illness in patients in the emergency department. Eur J Clin Invest 1997; 27: 977-81.

[9] Newman KP, Neal MT, Roberts M, Goodwin KD, Hatcher EA, Bhattacharya SK. The importance of lost minerals in heart failure. Cardiovasc Hematol Agents Med Chem 2007; 5: 295-9.

[10] Chhokar VS, Sun Y, Bhattacharya SK, *et al.* Hyperparathyroidism and the calcium paradox of aldosteronism. Circulation 2005; 111: 871-8.

[11] Tfelt-Hansen J, Hansen JL, Smajilovic S, Terwilliger EF, Hanso S, Seikh SP. Calcium receptor is functionally expresses in rat neonatal ventricular cardiomyocytes. Am J Physiol 2006; 290: H1165-71.

[12] Khouzam RN, Dishmon DA, Farah V, Flax SD, Carbone LD, Weber KT. Secondary hyperparathyroidism in patients with untreated and treated congestive heart failure. Am J Med Sci 2006; 331: 30-4.

[13] Lee AH, Mull RL, Keenan GF, *et al.* Osteoporosis and bone morbidity in cardiac transplant recipients. Am J Med 1994; 96: 35-41.

[14] Stefanelli T, Pacher R, Woloszczuk R, Glogar D, Kaindl F. Parathyroid hormone and calcium behavior in advanced congestive heart failure. Zts Kardiol 1992; 81: 121-5.

[15] Sun Y, Ahokas RA, Bhattacharya SK, Gerling IC, Carbone LD, Weber KT. Oxidative stress in aldosteronism. Cardiovasc Res 2006; 71: 300-9.

[16] Hagström E, Hellman P, Larsson TE, *et al.* Plasma parathyroid hormone and the risk of cardiovascular mortality in the community. Circulation 2009; 119: 2765-71.

[17] Rao RM, Yan Y, Wu Y. Dietary calcium reduces blood pressure, parathyroid hormone, and platelet cytosolic calcium responses in spontaneously hypertensive rats. Am J Hypertension 1994; 7: 1052-7.

[18] Uchimoto S, Tsumura K, Kishimoto H, Yamashita N, Morii H. Implication of parathyroid hormone for the development of hypertension in young spontaneously hypertensive rats. Miner Electrolyte Metabol 1995; 21: 82-6.

[19] Mann JF, Wiecek, A, Bommer J, Ganten U, Ritz E. Effects of parathyroidectomy on blood pressure in spontaneously hypertensive rats. Nephron 1987; 45: 46-52.

[20] Kishimoto H, Tsuzmura K, Fujioka S, Uchimoto S, Morii H. Chronic parathyroid hormone administration reverses the anti-hypertensive effect of calcium loading in young spontaneously hypertensive rats. Am J Hypertension 1993; 6: 234-40.

[21] Rybczynska A, Boblewski K, Lehmann A, *et al*. Calcimimetic NPS R-568 induces hypotensive effect in spontaneously hypertensive rats. AMJ Hypertension 2005; 18: 364-71.

[22] Stern N, Lee DB, Silis V, *et al*. Effects of high calcium intake on blood pressure and calcium metabolism in young SHR. Hypertension 1984; 6: 639-46.

[23] McCarron DA, Yung NN, Ugoretz BA, Krutzik S. Distrubances of calcium metabolism in the spontaneously hypertensive rat. Hypertension 1981; 3: 1162-7.

[24] Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D3 is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest 2002; 110: 229-38.

[25] Sigmund CD. Regulation of renin expression and blood pressure by vitamin D3. J Clin Invest 2002; 110: 155-6.

[26] Petrov V, Lijnen P. Modification of intracellular calcium and plasma rennin by dietary calcium in men. Am J Hypertension 1999; 12: 1217-24.

[27] DiPette DJ, Christenson W, Nickols MA, Nickols GA. Cardiovascular responsiveness to parathyroid hormone (PTH) and PTH-related protein in genetic hypertension. Endocrinology 1992; 130: 2045-51.

[28] Welsch S, Schordan E, Coquard C, *et al.* Abnormal renovascular parathyroid hormone-1 receptor in hypertension: Primary defect or secondary to angiotensin II type 1 receptor actuivation? Endocrinology 2006; 147: 4384-91.

[29] Santos S, Bosch RJ, Ortega A, *et al.* Up-regulation of parathyroid hormone-related protein in folic acid-induced acute renal failure. Kidney Int 2001; 60: 982-95..

[30] Bernini G, Moretti A, Lonzi S, Bendinelli C, Miccoli P, Salvetti A. Renin-angiotensin-aldosterone system in primary hyperparathyroidism before and after surgery. Metabolism 1999; 48: 298-300.

[31] Gennari C, Nami R, Gonelli S. Hypertension and primary hyperprarthyroidsm: the role of adrenergic and renin-angiotensin-aldosterone systems. Miner Electrolyte Metab 1995; 21: 77-81.

[32] Bogin E, Massry SG, Harary I. Effect of parathyroid hormone on rat heart cells. J Clin Invest 1981; 67: 1215-27.

[33] Schlüter KD, Piper HM. Trophic effects of catecholamines and parathyroid hormone on adult ventricular cardiomyocytes. Am J Physiol 1992; 263: H1739-46.

[34] Smogorzewski M, Zayed M, Zhang Y-B, Roe J, Massry SG. Parathyroid hormone increases cytosolic calcium concentration in adult rat cardiac myocytes. Am J Physiol 1993; 264: H1998-2006.

[35] Baczynski R, Massry SG, Kohan R, Magott M, Saglikes Y, Brautbar N. Effect of parathyroid hormone on myocardial energy metabolism in the rat. Kidney Int 1985; 27: 718-25.

[36] Motoyama HI, Friedman PA. Calcium-sensing receptor regulation of PTH-dependent calcium absorption by mouse cortical ascending limbs. Am J Physiol 2002; 283: F399-406.

[37] Schlüter KD, Weber M, Piper HM. Parathyroid hormone induce protein kinase C but not adenylate cyclase in adult cardiomyocytes and regulates cyclic AMP levels *via* protein kinase C-dependent phosphosdiesterase activity. Biochem J 1995; 310: 439-44.

[38] Schlüter KD, Wingender E, Tegge W, Piper HM. Parathyroid hormone-related protein antagonizes the action of parathyroid hormone on adult cardiomyocytes. J Biol Chem 1996; 271: 3074-8.

[39] Andersson P, Rydberg E, Willenheimer R. Primary hyperparathyroidism and heart disease –a review. Eur Heart J 2004; 25: 1776-87.

[40] Rostand SG, Drüecke TB. Parathyroid hormone, vitamin D, and cardiovascular disease in chronic renal failure. Kidney Int 1999; 56: 383-92.

[41] De Boer IH, Gorodetskaya I, Young B, Hsu C-Y, Chertow GM. The severity of secondary hyperparathyroidms in chronic renal insuffiency is GFR-dependent, race-dependent, and associated with cardiovascular disease. J Am Soc Nephrol 2002; 13: 2762-9.

[42] Nagashima M, Hashimoto K, Shinsato T, *et al.* Marked improvement of left ventricular function after parathyroidectomy in a he-

madialysis patient with secondary hyperparathyroidism and left ventricular dysfunction. Circ J 2003; 67: 269-72.

[43] Vestergaard P, Mosekilde L. Cohort study on effects of parathyroid surgery on multiple outcomes in primary hyperparathyroidism. Br Med J 2003; 327: 530-5.

[44] Kong CH, Farrington K. Determinants of left ventricular hypertrophy and its progression in high-flux haemodialysis. Blood Purif 2003; 21: 163-9.

[45] Schlier A, Schreckenberg R, Abdallah Y, *et al*. CGRP-α responsiveness of adult rat ventricular cardiomyocytes from normotensive and spontaneously hypertensive rats. Eur J Cell Biol 2009; 88: 227-41.

[46] Cueille C, Pidoux E, de Vernejoul M-C, Ventura-Clapier R, Garel J-M. Increased myocardial expression of RAMP1 and RAMP3 in rats with chronic heart failure. Biochem Biophys Res Commun 2002; 2945: 340-6.

[47] Ross G, Schlüter KD. Cardiac-specific effects of parathyroid hormone-related peptide: Modification by aging and hypertension. Cardiovasc Res 2005; 66: 334-44.

[48] Schreckenberg R, Wenzel S, da Costa Rebelo RM, Röthig A, Meyer R, Schlüter KD. Cell-specific effects of nitric oxide deficiency on parathyroid hormone-related peptide (PTHrP) responsiveness and PTH1 receptor expression in cardiovascular cells. Endocrinology 2009; 150: 3735-41.

[49] Rossouw JE, Anderson GL, Prentice RL, *et al*. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principle results from the Women's Health Initiative randomized controlled trial. JAMA 2002; 288: 321-33.

[50] Zaruba MM, Huber BC, Brunner S, *et al*. Parathyroid hormone treatment after myocardial infarction promotes cardiac repair by enhanced neovascularization and cell survival. Cardiovasc Res 2008; 77: 722-31.

[51] Schlüter KD, Maxeiner H, Wenzel S. Mechanisms that regulate homing function of progenitor cells in myocardial infarction. Minerva Cardiol 2009; 57: 203-17.

[52] Abdallah Y, Ross G, Dolf A, Heinemann MP, Schlüter KD. N-terminal parathyroid hormone-related peptide hyperpolarizes endothelial cells and causes a reduction of the coronary resistance of the rat heart *via* endothelial hyperpolarization. Peptides 2006; 27: 2927-34.

[53] Schlüter KD, Katzer C, Frischkopf K, Wenzel S, Taimor G, Piper HM. Expression, release, and biological activity of parathyroid hormone-related peptide from coronary endothelial cells. Circ Res 2000; 86: 946-51.

[54] Maeda S, Sutliff RL, Qian J, *et al*. Targeted overexpression of parathyroid hormone-related protein (PTHrP) to vascular smooth muscle in transgenic mice lowers blood pressure and alters vascular contractility. Endocrinology 1999; 140: 1815-25.

[55] Qian J, Lorenz NJ, Maeda S, *et al*. Reduced blood pressure and increased sensitivity of the vasculature to parathyroid hormone-related protein (PTHrP) in transgenic mice overexpressing the PTH/PTHrP receptor in vascular smooth muscle cells. Endocrinology 1999; 140: 1826-33.

[56] Fritsch S, Lindner V, Welsch S, *et al*. Intravenous delivery of PTH/PTHrP type 1 receptor cDNA to rats decreases heart rate, blood pressure, renal tone, renin angiotensin system, and stress-induced cardiovascular response. Am J Soc Nephrol 2004; 15: 2588-600.

[57] Degenhardt H, Jansen J, Schulz R, Sedding D, Braun-Dulläus R, Schlüter KD. Mechanosensitive release of parathyroid hormone-related peptide from cornary endothelial cells. Am J Physiol 2002; 283: H1489-96.

[58] Grohé C, van Eickels M, Wenzel S, *et al*. Sex-specific differences in ventricular expression and function of parathyroid hormone-related peptide. Cardiovasc Res 2004; 61: 307-16.

[59] Wenzel S, Schorr K, Degenhardt H, *et al*. TGF-β1 downregulates PTHrP in coronary endothelial cells. J Mol Cell Cardiol 2001; 33: 1181-90.

[60] Conzelmann C, Krasteva G, Weber K, Kummer W, Schlüter KD. Parathyroid hormone-related protein (PTHrP)-dependent regulation of bcl-2 and tissue inhibitor of metalloproteinase (TIMP)-1 in coronary endothelial cells. Cell Physiol Biochem 2009; 24: 493-502.

[61] Monego G, Arena V, Pasquini S, *et al*. Ischemic injury activates PTHrP and PTH1R expression in human ventricular cardiomyocytes. Basic Res Cardiol 2009; 104: 427-34.

[62] Giannakoulas G, Karvounis H, Koliakos G, *et al*. Parathyroid hormone-related protein is reduced in severe chronic heart failure. Peptides 2006; 27: 1894-7.

[63] Ogino K, Omura K, Kinugasa Y, *et al*. Parathyroid hormone-related protein is produced in the myocardium and increased in patients with congestive heart failure. J Clin Endocrinol Metab 2002; 87: 4722-7.

[64] Saussine C, Massfelder T, Parnin F, Judes C, Simeoni U, Helwig JJ. Renin stimulating properties of parathyroid hormone-related peptide in the isolated perfused rat kidney. Kidney Int 1993; 44: 764-73.

[65] Massfelder T, Taesch N, Fritsch S, *et al*. Type 1 parathyroid hormone receptor expression level modulates renal tone and plasma renin activity in spontaneously hypertensive rats. J Am Soc Nephrol 2002; 13: 639-48.

[66] Schlüter KD, Weber M, Piper HM. Effects of PTH-rP(107-111) and PTH-rP(7-34) on adult cardiomyocytes. J Mol Cell Cardiol 1997; 29: 3057-65.

[67] Massfelder T, Dann P, Wu TL, Vasavada R, Helwig JJ, Stewart AF. Opposing mitogenic and anti-mitogenic actions of parathyroid hormone-related protein in vascular smooth muscle cells: a critical role for nuclear targeting. Proc Natl Acad Sci USA 1997; 94: 13630-5.

[68] Schorr K, Taimor G, Degenhardt H, Weber K, Schlüter KD. Parathyroid hormone-related peptide is induced by stimulation of α1A-adrenoceptors and improves resistance against apoptosis in coronary endothelial cells. Mol Pharmacol 2003; 63: 111-8.

[69] Ross G, Heinemann MP, Schlüter KD. Vasodilatory effect of tuberoinfundibular peptide (TIP39): Requirement of receptor desensitization and its beneficial effect in the post-ischemic heart. Peptides 2007; 28: 878-86.

[70] Ross G, Engel P, Abdallah Y, Kummer W, Schlüter KD. Tuberoinfundibular peptide of 39 residues: A new mediator of cardiac function *via* nitric oxide production in the rat heart. Endocrinology 2005; 146: 2221-8.

[71] Shane E, Mancini D, Aaronson K, *et al*. Bone mass, vitamin D deficiency, and hyperparathyroidism in congestive heart failure. Am J Med 1997; 103: 197-207.

[72] Zittermann A, Schleithoff SS, Tenderich G, Berthold HK, Korfer R, Stehle P. Low vitamin D status: a contributing factor in the pathogenesis of congestive heart failure. J Am Coll Cardiol 2003; 41: 105-12.

[73] Kim DH, Sabour S, Sagar UN, Adams S, Whellan DJ. Prevalence of hypovitaminosis D in cardiovascular diseases. Am J Cardiol 2008; 102: 1540-4.

[74] Hsu CH, Patel S, Young EW. Calcemic response to parathyroid hormone in spontaneously hypertensive rats: role of calcitriol. J Lab Clin Med 1987; 110: 682-9.

[75] Simpson RU, Hershey SH, Nibbelink KA. Characterization of heart size and blood pressure in the vitamin D receptor knockout mouse. J Steroid Biochem Mol Biol 2007; 103: 521-4.

[76] Mancouso P, Rahman A, Hershley SD, Dandu L, Nibbelink KA, Simpson RU. 1,25-Dihydroxyvitamin-D3 treatment reduces cardiac hypertrophy and left ventricular diameter in spontaneously hypertensive heart failure-prone (cp/+) rats independent of changes in serum leptin. J Cardiocvasc Pharmacol 2008; 51: 559-64.

[77] McCarty MF. Nutritional modulation of parathyroid hormone secretion may influence risk for left ventricular hypertrophy. Med Hypothesis 2005; 64: 1015-21.

[78] Barnett AG, De Looper M, Fraser JF. The seasonality in heart failure and total cardiovascular deaths. Aust NZ J Public Health 2008; 32: 408-13.

[79] Zittermann A, Schleithoff SS, Koerfer R. Vitamin D insufficiency in congestive heart failure: Why and what to do about? Heart Fail Rev 2006; 11: 25-33.

[80] Weishaar RE, Simpson RU. Vitamin D3 and cardiovascular function in rats. J Clin Invest 1987; 79: 1706-12.

[81] Walters MR, Ilenchuk TT, Claycomb WC. 1,25-Dihydroxyvitamin D3 stimulates 45Ca2+ uptake by cultured adult rat ventricular cardiac muscle cells. J Biol Chem 1987; 262: 2536-41.

[82] Green JJ, Robinson DA, Wilson GE, Simpson RU, Westfall MV. Calcitriol modulation of cardiac performance *via* protein kinase C. J Mol Cell Cardiol 2006; 41: 350-9.

[83] Levin A, Li YC. Vitamin D and its analogues: do they protect against cardiovascular disease in patients with kidney disease? Kidney Int 2005; 68: 1973-81.

[84] Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr 2006; 83: 754-9.

[85] Schleithoff SS, Tittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Combining calcium and vitamin D supllementation is not superior to calcium supplementation alone in improving disturbed bone metabolism in patients with congestive heart failure. Eur J Clin Nutr 2008; 62: 1388-94.

[86] Law PH, Sun Y, Bhattacharya SK, Chhokar VS, Weber KT. Diuretics and bone loss in rats with aldosteronism. J Am Coll Cardiol 2005; 46: 142-6.

[87] Goodwin KD, Ahokas RA, Bhattacharya SK, Sun Y, Gerling IC, Webter KT. Preventing oxidative stress in rats with aldosteronism by calcitriol and dietary calcium and magnesium supplements. Am J Med Sci 2006; 332: 73-8.

[88] Wenzel S, Tastan I, Abdallah Y, Schreckenberg R, Schlüter KD. Aldosterone improves contractile function of adult rat ventricular cardiomyocytes in a non-acute way: potential relationship to the calcium paradox of aldostzeronism. Basic Res Cardiol 2010; 105(2): 247-56.

Received: September 29, 2009 Revised: October 30, 2009 Accepted: November 30, 2009

© Schreckenberg *et al.;* Licensee *Bentham Open.*

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.