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A Role of Parathyroid Hormone for the Activation of Cardiac Fibroblasts in Uremia[1]

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ABSTRACT

Intermyocardiocytic fibrosis, *i.e.*, nonreparative interstitial fibrosis with collagen fiber deposition, is commonly found in uremic patients and animals. The volume density of interstitial tissue in the left papillary muscle of uremic animals was found to be increased (from 1.9 ± 0.7 to 4.2 ± 1.1%; $P < 0.001$). The nuclei of interstitial cells, but not of endothelial cells, were enlarged, pointing to an activating signal that specifically acts on interstitial cells. Because of the known action of parathyroid hormone (PTH) on the heart, a potential role of PTH in the genesis of fibrosis was explored by comparing subtotally nephrectomized (NX) parathyroidectomized (PTX) rats receiving by osmotic minipump either saline or rat 1,34 PTH (100 ng/kg per hour dissolved in NaCl). Animals were on a standard 0.95% Ca diet. After PTX, they were switched to a high-calcium (3%) diet. At the end of the 14-day experiments, NX-PTX-PTH animals and NX-PTX-solvent animals were comparable with respect to mean body weight (335 versus 338 g), serum creatinine (1.2 versus 1.2 mg/dL), and serum-Ca (2.66 versus 2.63 mmol/L). The volume densities of cardiac interstitium were 4.71 ± 0.87 versus 1.49 ± 0.49, and those of capillaries were 8.07 ± 1.54 versus 7.94 ± 2.62, respectively ($P < 0.001$ by analysis of variance). Thus, PTX abolished and PTH restored intermyocardiocytic changes of experimental uremia. These observations argue for a permissive role of PTH for fibroblast activation and the genesis of the cardiac fibrosis of uremia.

Key Words: *Uremia, parathyroid hormone, cardiac fibrosis, diastolic heart malfunction*

Interstitial cardiac fibrosis has long been known to be associated with uremia (1,2). This finding has recently been rediscovered, and interstitial cardiac fibrosis was demonstrated both in experimental studies (3) and in postmortem material (4). Interstitial fibrosis of the heart may be important in determining left ventricular (LV) compliance (5), systolic stress/strain relationship (6), and electrical properties of the heart (7). Both direct (8) and indirect (9–11) measurements suggest impaired diastolic filling properties of the LV of uremic patients. This may, at least in part, be related to fibrosis with deposition of collagen fibers (12).

In the heart of uremic animals, the volume densities of the cytoplasm and nucleus were increased in interstitial cells, but not in endothelial cells (3). Furthermore, in extracardiac organs, *e.g.*, the liver or pancreas, no fibrosis was seen. This finding raised the possibility that some specific signal selectively acted to activate interstitial cells in the heart. We made the (unpublished) chance observation that increased volume density of the interstitium was not observed in parathyroidectomized (PTX) rats with subtotal nephrectomy.

To pursue this lead in a formal manner, we compared the hearts of eucalcemic subtotally nephrectomized PTX rats given either solvent or the amino-terminal 1,34 fragment of rat parathyroid hormone (PTH) by osmotic minipump. Using stereologic techniques, we could reconfirm our previous finding of increased interstitial volume in the heart of uremic animals. We now show that such an increase combined with the ultrastructural evidence of fibroblast activation is seen only in the presence of PTH.

MATERIAL AND METHODS

Animals and Protocol

Male Sprague Dawley rats (300 g; Invanovas Co., Kisslegg, Germany) were housed in single cages at constant room temperature (20°C) and humidity (25%) under a controlled light on/off cycle.

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After a 3-day adaptation period, the animals were divided into four groups by random numbers. The left kidney was resected with the rat under ether anesthesia (resection of upper and lower kidney poles leaving an intact kidney segment in between). After another 14 days, the right kidney was removed. Concomitantly, control animals were sham operated (decapsulation of the kidney). Care was taken that the adrenals were not damaged. Three days after subtotal nephrectomy, surgical resection of the parathyroid (PT) was performed under microscopical control. The respective control animals were sham operated i.e., sham PTH. Six days after PTX/sham operation, the infusion of solvent or PTH was started. Osmotic minipumps (Alzet; 2 mL 2, flowrate: 5.0 ± 0.75 μL/h) were implanted sc. The PTX/solvent group received 0.9% NaCl, whereas the PTX/PTH group received 1,34 rat PTH dissolved in 0.9% NaCl at a dose of 100 ng/kg per hour (Bissendorf Biochemicals Co., Hannover, Germany; Lot No. 049).

The animals in all groups were fed a diet containing 0.95% calcium, 0.75% Pi, and 500 U vitamin D_3/kg (Altromin Co., Lage/Lippe, Germany) throughout the experiment, with the exception of the PTX animals receiving solvent. In this group, the standard diet was given until the beginning of infusion (Day 23; Figure 1). From Day 23 onward, this group of animals was fed a diet with a high calcium content, *i.e.*, 3% Ca, 0.75% Pi, and 500 U of vitamin D_3/kg (Altromin Co.), which had been shown in a pilot experiment to maintain eucalcemia. The animals were not pairfed and had free access to ionized water. Blood samples were taken from the tail vein. The experiment terminated on the 14th day after the implantation of the osmotic minipump. Blood pressure was measured by tail plethysmography.

Tissue Preparation

At the end of the experiment, the inferior abdominal aorta was catheterized and the viscera were fixed by retrograde vascular perfusion at a pressure of 110 mm Hg. Before fixation, the vascular system was flushed with dextran solution (Rheomacrodex®) containing 0.5 g/L procain-HCL for 2 min. Ten seconds after the beginning of the aortic perfusion, the vena cava inferior was incised to drain the blood. After dextran was given to avoid capillary collapse from low venous pressure, the vascular system was subsequently perfused with 0.2 M phosphate buffer containing 3% glutaraldehyde for 12 min. After completion of the perfusion, the heart of each animal was excised for determination of weight and volume. LV papillary muscles were randomly cut with a tissue sectioner, both longitudinally and transversely, as described elsewhere (13), and used for stereologic measurements. The right and left ventricles were assessed semiquantitatively as were liver and pancreas. At least two transversely cut 200-μm slices and two longitudinally cut 200-μm slices were randomly selected for stereology and embedded in Epon-Araldite. Semithin sections (1 μm) were stained with methylene-blue and basic fuchsin (14) and examined by light microscopy with oil immersion and phase contrast at a magnification of 1,000:1. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with Zeiss EM 10 electron microscope (Zeiss Co., Oberkochen, FRG). Several transversely embedded probes per animal were chosen for ultrathin sectioning and were qualitatively analyzed.

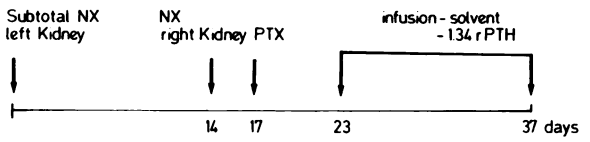

Figure 1. Protocol of the experiment. NX, nephrectomy. r, rat.

Quantitative Stereology

Stereologic analysis was performed on transverse and longitudinal sections of the left ventricle papillary muscle at a magnification of 1,000:1.

Stage 1 (Light Microscope, Magnification 1,000:1). Eight systematically subsampled test areas per section (57,600 μm^2) were analyzed with a Zeiss eyepiece containing 100 points and 10 lines (total length, 900 μm). The points were used for point counting. Capillary profiles as well as the volume density of the nonvascular myocardial interstitium and the volume density of the myocytes were counted. Reference volume was the total myocardial tissue of the LV papillary muscle. Volume density (V_v), *i.e.*, volume of the structure under study (in cubic centimeters) per unit tissue volume (in cubic centimeters) was obtained by point counting according to the equation $P_p = V_v$ where P_p is point density, *i.e.*, point number of profiles per total point number.

Stage 2 (Electron Microscopy, Magnification 10,000:1). Several ultrathin sections of the LV papillary muscle per animal were semiquantitatively investigated.

Statistics

Data are given as mean ± SD. Differences between the groups were evaluated by analysis of variance followed by the Scheffé test. Results were considered significant when the probability of error was $P < 0.05$.

RESULTS

Animal Data

No significant differences were seen between the two PTX groups with respect to LV weight or LV

TABLE 1. Animal data[a]

	Body Wt (g)	Blood Pressure (mm Hg)	LV Wt (g)	LV/Body Wt $\times 10^3$	Right Ventricle Wt (g)	Serum Creatinine (mg/dL)	Serum Urea (mg/dL)	Serum calcium (mmol/L)
(1) Sham operated, PT intact (N = 10)	332 ± 29		0.89 ± 0.15	2.69 ± 0.444	0.215 ± 0.043	0.30 ± 0.07	30.0 ± 8	2.56 ± 0.12
(2) Uremia, PT intact (N = 8)	337 ± 26	151 ± 6.4	0.83 ± 0.14	2.57 ± 0.382	0.202 ± 0.024	1.1 ± 0.1	149 ± 31	2.60 ± 0.13
(3) Uremia, PTX + Solvent (N = 9)	338 ± 20	152 ± 5.1	0.91 ± 0.08	2.71 ± 0.253	0.249 ± 0.051	1.2 ± 0.3	151 ± 33	2.66 ± 0.13
(4) Uremia, PTX + 1,34 Rat PTH (N = 8)	335 ± 22	141 ± 4.8	0.84 ± 0.06	2.52 ± 0.179	0.220 ± 0.016	1.2 ± 0.2	156 ± 33	2.63 ± 0.13
Analysis of variance, Scheffé test	NS	NS	NS	NS	NS	$P < 0.001$ 2–3, NS[b] 2–4, NS[b]	$P < 0.001$ 2–3, NS[b] 2–4, NS[b]	NS

[a] Values are mean ± SD. NS, not significant.
[b] Scheffé comparisons.

weight/body weight ratio (weights after perfusion fixation are not necessarily indicative of *in vivo* weights) (Table 1). The animals were moderately uremic and had comparable serum calcium concentrations on 0.95% calcium diet in the PTH-treated versus 3.0% Ca in the solvent-treated PTX animals. Blood pressure was also not different between the three groups.

Stereologic Measurements in the Myocardium

As shown in Table 2, a significant increase of nonvascular (noncapillary) interstitial space was seen in uremic PT-intact animals. Such an increase was obliterated by PTX but was restored by the infusion of 1,34 rat PTH (rPTH) in eucalcemic animals. There was a tendency for an inverse decrease in the volume density of capillaries, but the difference was not statistically significant. The volume density of the cardiomyocytes remained unchanged.

Semiquantitative scoring (data not given) showed that the changes were homogenously distributed throughout the heart occurring in both the left and right ventricles. An analysis of the ultrastructure (Figure 2) showed signs of activation of interstitial cells in the uremic PTX animals with the infusion of 1,34 rPTH, but not in uremic PTX animals. These changes comprised nuclear swelling, enlarged cytoplasm, activation of the Golgi apparatus, and extrusion of collagen fibers into the interstitial space. Significantly, no changes in the ultrastructure of the endothelial cells were noted. Calcium deposits in the interstitium were absent, as was evidence of mito-

TABLE 2. Stereologic measurements—effect of uremia and PTH

	Volume Density of Nonvascular Interstitial Space (Vol %)	Volume Density of Capillaries (Vol %)	Volume Density of Myocytes (Vol %)
(1) Sham Operated PT Intact (N = 8)	1.36 ± 0.55	9.13 ± 1.45	89.6 ± 1.41
(2) Uremia, PT Intact (N = 8)	4.46 ± 0.74	7.12 ± 2.09	88.5 ± 2.69
(3) Uremia, PTX + Solvent (N = 8)	1.49 ± 0.49	7.94 ± 2.62	88.7 ± 2.64
(4) Uremia, PTX + 1,34 Rat PTH (N = 11)	4.71 ± 0.87	8.07 ± 1.54	87.6 ± 1.44
Analysis of Variance	$P < 0.001$	NS	NS
Scheffé Test	1–2, $P < 0.001$[b] 1–3, NS[b] 1–4, $P < 0.001$[b] 2–4, NS[b] 2–3, $P < 0.001$[b] 3–4, $P < 0.001$[b]		

[a] Values are mean ± SD. NS, not significant.
[b] Scheffé comparisons.

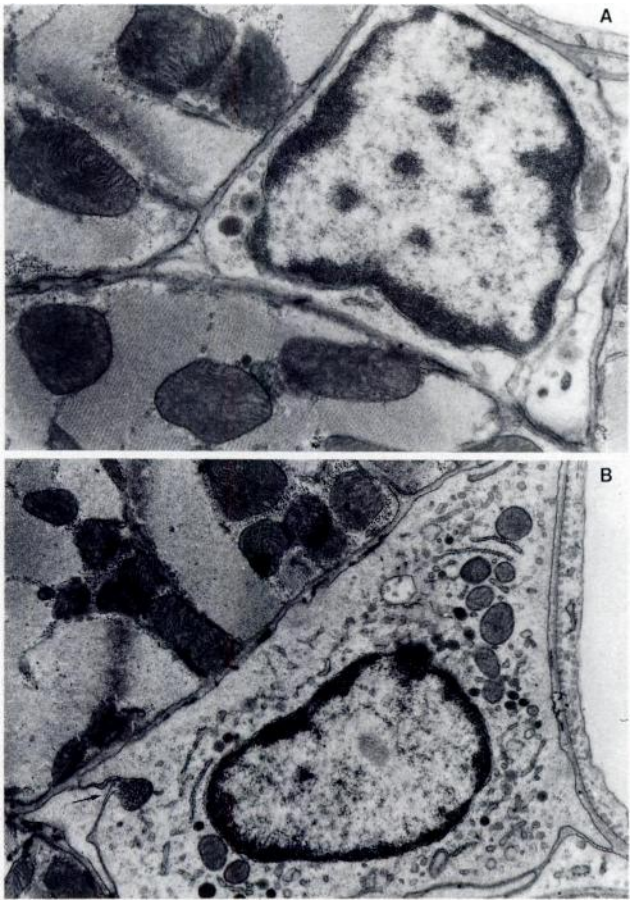

Figure 2. Ultrastructure of interstitial cells of the myocardium. Comparison of solvent-infused (a, ultrathin section; magnification, 3,000:1) and 1,34 rPTH-infused (b, ultrathin section; magnification, 24,000:1) animals. Note the interstitial cell of a PTH-infused animal with nuclear swelling, enlarged cytoplasm, activation of the Golgi apparatus, and invagination of the cell membrane (arrow); the invagination contains collagen fibers in the extracellular space. This was not seen in nonuremic control animals.

chondrial calcium overload. The deposition of calcium in the heart or in extracardiac viscera, *e.g.*, the lung, was also excluded with the Kossa stain. To evaluate whether the changes are specific for the heart, we examined hepatic and pancreatic tissue using a semiquantitative scoring system (data not given); no significant changes in the width of the interstitia were seen after subtotal nephrectomy or manipulation of the PTH status, respectively.

DISCUSSION

This study in rats with short-term uremia documents that PT status, independent of calcemia, is a factor in the characteristic selective activation of cardiac fibroblasts and in the genesis of incipient intermyocardiocytic fibrosis with collagen deposition (1–4). Increased volume density of the cardiac interstitium confirms previous observations in this laboratory in rats with uremia of moderate duration (4,12). In uremia of 14-mo duration (12), interstitial activation had progressed to dense collagen deposition in the cardiac interstitium. Expansion in the myocardium interstitium cannot be explained by edema. The ratio of dry/wet weight is not altered in animals subtotally nephrectomized by the above protocol (sham-operated controls, cardiac dry weight/wet weight, 0.246 ± 0.002 versus 0.239 ± 0.023 in nephrectomized rats). Although modest edema can be documented by measurements of impedance (unpublished observations), ultrastructural analysis shows that cell swelling, *i.e.*, activation, is the major cause of interstitial expansion. Although the magnitude of the difference in interstitial volume density was small, the measurements were highly reproducible and the difference was highly significant by analysis of variance. Measurements of PT weight and morphometric analysis showed that, in short-term uremia, the PT glands are activated in PT-intact animals (15). Because of species differences, an increase in circulating PTH levels is difficult to verify in the absence of a rat-specific test system. We emphasize that we infused rat 1,34 PTH to avoid any species-related problems that might confound the results.

In the past, several investigators observed that cardiac changes in renal failure were related to PTH concentration (16,17). "Inadequate" LV hypertrophy was noted in uremic patients with high PTH levels, suggesting that PTH interfered with the development of LV hypertrophy. A similar tendency was noted in our study (Table 1); possibly because of the small sample size, the differences were not statistically significant. The heart is known to be a target organ for PTH, causing increased beating frequencies in cell cultures (18), positive inotropic effects (19), and cellular calcium overload with ensuing cardiomyocytic metabolic disturbances (20). The latter could be prevented by the administration of the calcium channel blocker verapamil and was thought to be related to cellular calcium overload. In this context, it is of note that neither intracellular nor extracellular calcium deposits were noted in our short-term studies.

The mechanism(s) through which PTH causes the interstitial cardiac changes in experimental uremia remain(s) to be clarified. Cardiac fibrosis has not been described in patients with primary hyperparathyroidism; this is confirmed by our own unpublished observations. Such negative observation does not exclude, however, that PTH exerts a permissive effect in the presence of uremia. Closer inspection of our results show that PTX (*i.e.*, PTX versus solvent) did largely, but possibly not completely, reverse the

increase in volume density of the nonvascular interstitial space. This parameter was somewhat higher in nephrectomized/PTX/solvent animals than in sham-operated PT-intact animals. This may merely denote that PTH is only one permissive factor among others. Brilla *et al.* (21) showed that aldosterone is also a factor related to the genesis of cardiac fibrosis and that aldosterone levels are elevated in renal failure. Confirming our previous results (4), ultrastructural evidence of cell activation was restricted to interstitial cells and was not seen in endothelial cells. This argues for a signal specifically acting on fibroblasts. It is unknown whether cardiac interstitial fibroblasts have PTH receptors and PTH-dependent adenylate cyclase or Pi response, respectively. The effect of PTH seems to be specific for the heart, because no PTH-dependent changes of interstitial width were observed in the liver and in the pancreas. It is known that endothelial cells interact with periendothelial cells via cellular cross-talk. In this context, it is of interest that no ultrastructural changes were noted in capillary endothelial cells, although this obviously does not exclude more subtle mechanisms.

The result that PTH promoted cardiac fibrosis may seem paradoxical in view of the known suppression of collagen synthesis in fetal rat calvaria (22). In agreement with previous observations (12), it is of note, however, that we observed no fibrosis in extracardiac interstitial tissue, so that changes appear to be quite selective for the heart. Furthermore, PTH has been shown to induce collagenase in rat osteoblasts on a transcriptional level (23). Although our study did not assess whether the incipient accumulation of collagen is more related to increased synthesis or diminished breakdown of collagen, our ultrastructural findings clearly suggest that increased collagen deposition is responsible, at least in part. We emphasize that in this model of short-term uremia, such dense interstitial fibrosis as described previously (12) is not observed, although collagen deposition was clearly demonstrable by electron microscopy. Direct cellular activation by PTH, as one might postulate on the basis of our experiments, would not be without precedents. Whitfield *et al.* (24) noted that PTH activates T lymphocytes. Furthermore, PTH has been shown to exert a great variety of effects on nonclassic target organs (20,25). Indirect effects of PTH must also be considered. Brilla *et al.* found a permissive role of aldosterone on the development of cardiac interstitial fibrosis (21). It is therefore of note that Olgaard *et al.* (26) found a permissive role of PTH on adrenal aldosterone secretion.

In principle, cardiac fibrosis may be caused by different mechanisms. One can distinguish between primary fibrosis and reparative fibrosis (27). In our short-term experiment, we saw no calcium deposits, no cardiomyocyte necrosis, and no replacement fibrosis. Although prolonged action of PTH may cause cellular calcium overload and sensitize cells to catecholamines, we conclude that cardiac fibrosis in our short-term experiments is of the reactive or primary type and not of the reparative type. It is unlikely that the development of cardiac fibrosis was caused by primary hemodynamic factors. First, in a previous study (28), rats with renovascular hypertension were examined and their LV/body weight ratio ($3.78 \pm 0.56 \times 10^3$) clearly exceeded that of the animals in this study (*e.g.*, Table 1, 2.77). Nevertheless, in these animals, only borderline interstitial expansion was noted (3.0 ± 1.2 versus 2.4 ± 0.5 in controls compared with 4.46 ± 0.74 in PT-intact uremic animals of this study; Table 2). A hemodynamic explanation is further rendered unlikely by the fact that blood pressure were similar in the three experimental uremic groups as were weight/body weight ratios. The hypothesis of a nonhemodynamic origin is further supported by the observation that similar findings were seen in the right and left ventricle. Although this observation does not provide direct proof to exclude hemodynamic factors, it has been argued that right cardiac involvement is more compatible with a metabolic than with a hemodynamic origin of the changes, *e.g.*, in hyperrenenemic states (27).

Cardiac fibrosis may have some important clinical consequences. Diastolic LV dysfunction is commonly noted in uremic patients (29,30), whereas systolic pumping function—in contrast to previous opinion—is normal, at least in the absence of ischemic heart disease (31). Undoubtedly, several factors play a role in diastolic LV malfunction, *e.g.*, LV hypertrophy and impaired LV relaxation, but interstitial cardiac fibrosis may be another causal factor. No clinical studies are available to indicate whether diastolic LV function is more markedly impaired in uremic patients with severe hyperparathyroidism. The above observation adds another and novel effect of PTH to the long list of pathways through which this hormone may cause cardiocirculatory dysfunction in renal failure.

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REFERENCES

1. **Rössle R:** Über die serösen Entzündungen der Organe. Virchows Arch 1943;311:252–284.
2. **Langendorf R, Pirani CL:** The heart in uremia. Am Heart J 1947;33:282–307.
3. **Mall G, Rambausek M, Neumeister A, Kollmer S, Vetterlein F, Ritz E:** Myocardial interstitial fibrosis in experimental uremia—implications

for cardiac compliance. Kidney Int 1988;33: 804–811.
4. **Mall G, Huther W, Schneider J, Lundin P, Ritz E:** Diffuse intermyocardiocytic fibrosis in uremic patients. Nephrol Dial Transplant 1990;5:39–44.
5. **Weber KT, Janicki JS, Shroff SG, Pick R, Chen RM, Bashey RI:** Collagen remodeling of the pressure-overloaded, hypertrophied nonhuman primate myocardium. Circ Res 1988;62:757–765.
6. **Robinson TF, Cohen-Gould L, Factor SM:** Skeletal framework of mammalian heart muscle. Arrangement of inter- and pericellular connective tissue structures. Lab Invest 1983;49:482–498.
7. **Bakth S, Arena J, Lee W, *et al.*:** Arrhythmia susceptibility and myocardial composition in diabetes. J Clin Invest 1986;77:382–395.
8. **Kramer W, Wizemann V, Lämmlein G, *et al.*:** Cardiac dysfunction in patients on maintainance hemodialysis. II. Systolic and diastolic properties of the left ventricle assessed by invasive methods. Contrib Nephrol 1986;52:110–124.
9. **Ruffmann K, Mandelbaum A, Bomer J, Schmidli M, Ritz E:** Dopllerechocardiographic findings in dialysis patients. Nephrol Dial Transplant 1990;5:93–97.
10. **D'Cruz IA, Bhatt GR, Cohen HC, Glick G:** Echocardiographic detection of cardiac involvement in patients with chronic renal failure. Arch Intern Med 1978;138:720–724.
11. **London GM, Guérin AP, Marchais SJ, Métivier F:** Cardiomyopathy in end-stage renal failure. Semin Dial 1989;2:102–107.
12. **Amann K, Wiest G, Zimmer G, Gretz N, Ritz E, Mall G:** Reduced capillary density in the myocardium of uremic rats—a stereological study. Kidney Int 1992;42:1078–1084.
13. **Mall G, Schikora I, Mattfeldt T, Bodle R:** Dipyridamole induced capillary neoformation in the rat heart—a quantitative stereological study. J Lab Invest 1987;57:86–93.
14. **Di Saint Agnese PA, De Mesy Jensen KL:** Dibasic staining of large epoxy sections and applications to surgical pathology. Am J Pathol 1984;80:25–29.
15. **Szabo A, Merke J, Beier E, Mall G, Ritz E:** $1,25(OH)_2D_3$ inhibits parathyroid cell proliferation in experimental uremia. Kidney Int 1989;35:1049–1056.
16. **Bogin E, Levi J, Harary I, Massry SG:** Effect of parathyroid hormone on oxidative phosphorylation of heart mitochondria. Miner Electrolyte Metab 1982;7:151–156.
17. **London GM, de Verrejoul M-C, Fabiani F, *et al.*:** Secondary hyperparathyroidism and cardiac hypertrophy in hemodialysis patients. Kidney Int 1987;32:900–907.
18. **Bogin E, Massry SG, Harary I:** Effect of parathyroid hormone on rat heart cells. Lab Invest 1981;76:1215–1227.
19. **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;27:718–725.
20. **Klinger M, Alexiewicz JM, Linker-Israeli, *et al.*:** Effect of parathyroid hormone on human T cell activation. Kidney Int 1990;37:1543–1551.
21. **Brilla CG, Zhou G, Weber KT:** Aldosterone-mediated stimulation of collagen synthesis in cultured cardiac fibroblasts. J Hypertension 1992;10[Suppl 4]:S7.
22. **Kream BE, Rowe DW, Gworek SC, Raisz LG:** Parathyroid hormone alters collagen synthesis and procollagen mRNA levels in fetal rat calvaria. Proc Natl Acad Sci USA 1980;77:5654–5658.
23. **Quinn CO, Scott DK, Brinckerhoff CE, Matrisian LM, Jeffrey JJ, Partridge NC:** Rat collagenase. Cloning, amino acid sequence, and parathyroid hormone regulation in osteoblastic cells. J Biol Chem 1990;265:22342–22347.
24. **Whitfield JF, Mac Manns JP, Rixon RH:** The possible mediation by cyclic AMP of parathyroid hormone-induced stimulation of mitotic activity and deoxyribonucleic acid synthesis in rat thymic lymphocytes. J Cell Physiol 1970;75: 213–224.
25. **Urena P, Lee K, Weaver D, *et al.*:** PTH-PTHrP receptor mRNA expression as assessed by northern blot and in situ hybridization. [Abstract 102] J Bone Miner Res 1992;7[Suppl 1].
26. **Olgaard K, Dangaard H, Egfjord M.** Enhancement of the stimulatory effect of Ca^{2+} on aldosterone secretion by PTH. In Massry SG, Fujita T, Eds. New Actions of Parathyroid Hormone. New York: Plenum Press; 1989;265–270.
27. **Weber KT, Brilla CG:** Pathologic hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. Circulation 1991; 83:1849–1865.
28. **Mall G, Klingel K, Baust H, *et al.*:** Synergistic effects of diabetes mellitus and renovascular hypertension on the rat heart—stereological investigations on papillary muscles. Virchows Arch A 1987;411:531–542.
29. **Ritz E, Ruffmann K, Rambausek M, Mall G, Schmidli M:** Dialysis hypotension—is it related to diastolic left ventricular malfunction? Nephrol Dial Transplant 1987;2:293–297.
30. **Rostand SG, Sanders C, Kirk KA, Rutsky EA, Fraser RG:** Myocardial calcification and cardiac dysfunction in chronic renal failure. Am J Med 1988;85:651–657.
31. **Parfrey PS, Harnett JD, Griffiths SM, *et al.*:** The clinical course of left ventricular hypertrophy in dialysis patients. Nephron 1990;55:114–120.