RENAL AND CARDIOVASCULAR HYPERTROPHY: IMPLICATIONS FOR THE TREATMENT OF PATIENTS WITH HYPERTENSION, RENAL AND CARDIAC DISEASE

Robert J. Alpern, M.D.

Department of Internal Medicine

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INTRODUCTION

I would like to start this Grand Rounds with presentations of three patients. Patient 1 is a 45 year old African American male who presents with high blood pressure, 180/110, of unknown duration. Evaluation is noteworthy for left ventricular hypertrophy with strain on electrocardiogram, and a thickened left ventricular wall on echocardiogram. Patient #2 is a 20 year old Caucasian male with insulin-dependent diabetes mellitus. Evaluation is noteworthy for microalbuminuria, and enlarged kidneys on ultrasound. Patient #3 is a 30 year old Caucasian female who recovered from a prolonged episode of acute tubular necrosis one year ago. Her serum creatinine is 2.5 mg/dl and she excretes 750 mg/day of albumin in her urine. Blood pressure is 150/100.

All three of these patients have a number of features in common. First, all of these patients have common clinical disorders with which medical internists deal frequently. Second, in all of these patients cell hypertrophy is developing. In patients 1 and 3, hypertension is present, leading to hypertrophy of vascular smooth muscle cells and cardiac myocytes. In patient 2, diabetes mellitus has led to renal hypertrophy. In patient #3, a previous episode of acute tubular necrosis has led to nephron dropout, with the remaining degree of renal function attributable to hyperfunction and hypertrophy of remaining nephrons. Thus, cell hypertrophy is common, occurring in a number of common clinical situations. It therefore is important to understand the clinical significance of hypertrophy.

Hypertrophy refers to cell growth involving an increase in cell size. There is no increase in cell number. Thus, while protein synthesis is increased in hypertrophying cells, there is no change in DNA synthesis. In contrast, hyperplasia refers to cell growth with an increase in cell number. In these cells, there is synthesis of protein and DNA.

In response to the requirement for increased work, cells tend to undergo either hypertrophy or hyperplasia. Cardiac myocytes in adults are unable to undergo hyperplasia, and thus are forced to hypertrophy. Renal tubular cells, similarly, will tend to undergo hypertrophy in response to a requirement for increased function. However, in certain circumstances such as acute tubular necrosis, the cells are capable of hyperplasia. Similarly, vascular smooth muscle cells will hypertrophy in response to an increase in blood pressure. However, if a vessel wall is damaged they are able to undergo hyperplasia. Other cells are more likely to undergo hyperplasia. Thus, partial hepatectomy leads to hyperplasia of hepatocytes. Similarly, hematopoietic cells and lymphocytes will undergo hyperplasia in response to a demand for increased function.

In this grand rounds I will concentrate on cardiovascular and renal hypertrophy. There has been much research in these areas, and as noted above, hypertrophy of these organs occurs commonly. Cardiac hypertrophy occurs in any condition in which afterload is increased for a sustained period of time. Such conditions include hypertension, coarctation of the aorta, and aortic stenosis. In patients with these disorders, left ventricular wall thickness is increased, and the size of cardiac myocytes is increased. Hypertrophy also occurs with loss of cardiac mass. Thus, following myocardial infarction there is a decrease in the number of myocytes. In order to maintain function, remaining myocytes hypertrophy. Cardiac hypertrophy also occurs in high output states such as thyrotoxicosis and in exercise training. However, these latter types of hypertrophy appear to be different from that which occurs with increased afterload. This will be discussed below.

Vascular smooth muscle cells hypertrophy in response to hypertension. All forms of hypertension involve vasoconstriction, and chronic vasoconstriction is associated with an

increased size of vascular smooth muscle cells.

Renal hypertrophy occurs in conditions with loss of kidney mass. Thus, agenesis of one of the kidneys, ablation of a kidney, or nephron dropout due to parenchymal renal disease, will all lead to hypertrophy of the remaining nephrons. Renal hypertrophy has also been seen in diabetic nephropathy, where it has been postulated to contribute to its genesis. Lastly, renal hypertrophy is seen in chronic metabolic acidosis and chronic potassium deficiency. The clinical significance of hypertrophy in these latter conditions remains unclear.

HYPERTROPHY: FRIEND OR FOE?

A key issue is whether hypertrophy is beneficial or detrimental to the patient. There is little doubt that hypertrophy is beneficial in the short term. Cardiac hypertrophy likely improves the ability of the heart to pump against an increased afterload. In the presence of decreased renal mass, remaining nephrons develop higher single nephron glomerular filtration rates and higher rates of tubular transport. This is likely aided by the presence of hypertrophy in the remaining nephrons. The major issue is the long term effect of hypertrophy.

Heart

In the case of cardiac hypertrophy a number of stages can be considered. First, there is an initial hemodynamic overload. This initiates a hypertrophic response, resulting in compensation. This compensation phase, however, is later followed by a decompensation, or broken compensation phase (Katz, 1990). The question is whether hypertrophy contributes to this decompensation, or whether indeed it serves to prevent the decompensation, and other features of the hemodynamic overload are leading to the decompensation.

Table 1.
FUNCTIONAL CONSEQUENCES OF CARDIAC HYPERTROPHY

1.	Arrhythmias Conduction inhomogeneities Prolongation of the action potential
2.	Relaxation abnormalities Collagen Active processes (Ca reuptake)
3.	Vulnerability to hypoxic injury
4.	? Decreased contractility and decompensation Collagen Isoform switching

Table 1 lists some of the functional consequences of cardiac hypertrophy (Katz, 1990). First, cardiac hypertrophy is known to lead to arrhythmias. This is thought to be secondary to conduction inhomogeneities related to the altered anatomy and to a prolongation of the action potential. There is also a relaxation abnormality in cardiac hypertrophy which prevents normal filling of the ventricle during diastole. This may be partially structural in nature but likely also involves active processes. The hypertrophied left ventricle is also more vulnerable to hypoxic injury (Canby and Tomanak, 1990). Lastly, the question of whether hypertrophy leads to eventual decompensation and decreased contractility is controversial. As hypertrophy is associated with increased collagen synthesis, this may lead to decreased contractility (Caspari et al, 1977; Weber et al, 1988). In addition, as extensively studied in the rat, hypertrophy in response to increases in afterload is associated with isoform switching in the myosin heavy chain which can lead to decreases in cardiac contractility (see

below).

Vascular Smooth Muscle Cells

With regard to hypertension, vascular smooth muscle cell hypertrophy is clearly detrimental. Chronic hypertension is generally associated with hypertrophy of vascular smooth muscle cells. This hypertrophy may merely be secondary to the chronic increase in blood pressure, or may be induced by the pressor mechanisms that caused the hypertension. In any case, vascular smooth muscle cell hypertrophy is likely associated with an increased capacity for vasoconstriction. This would then contribute to a greater difficulty in treating high blood pressure (Folkow, 1978).

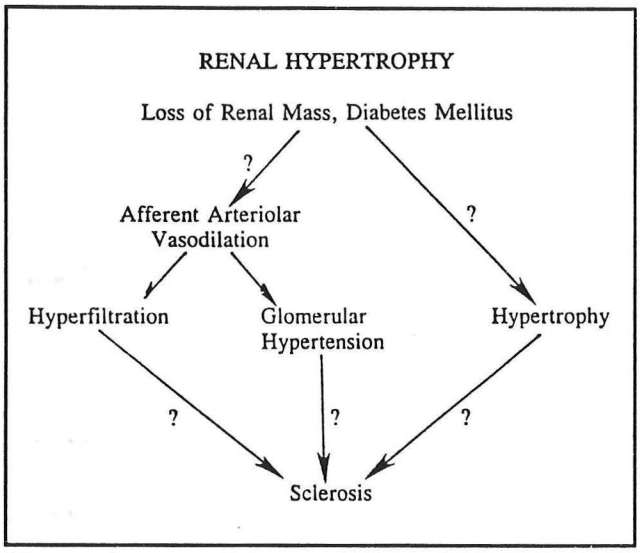


Figure 1

Kidney

The significance of renal hypertrophy has been extensively investigated with regard to loss of renal mass and diabetes mellitus (Figure 1). The response of the kidney to a loss of renal mass has been best studied in rats receiving a 1-2/3 nephrectomy. Segmental renal artery branches to 2/3 of one kidney are tied off, and the animal allowed to recover. At a later date, the opposite kidney is removed. The remaining nephrons then develop an increased single nephron glomerular filtration rate that serves to minimize the degree of loss of renal function. This adaptation involves afferent arteriolar vasodilation leading to increases in single nephron glomerular blood flow, glomerular filtration rate, and glomerular capillary pressure. While this response is associated with an initial improvement in renal function, over the long term it is associated with glomerular and interstitial sclerosis (see below). Loss of renal mass is also associated with hypertrophy of the kidney, which may also play a role in the sclerosis. As shown in **Figure 1**, there are many question marks as to cause and effect. Thus, it is unclear how loss of renal mass leads to afferent arteriolar vasodilation or hypertrophy. In addition, the relationship between hyperfiltration, glomerular hypertension, and hypertrophy, and sclerosis is not completely settled. A similar sequence of events occurs in diabetic nephropathy.

Table 2 shows results from the original classic study of Hostetter et al (1981). One and two-thirds nephrectomy was noted to cause an increase in single nephron GFR (SNGFR), in single nephron blood flow (Q_A), and in glomerular capillary pressure (P_{GC}). If rats undergoing 1-2/3 nephrectomy were placed on a low protein diet, all of these hemodynamic changes were reversed back to normal. Although dietary protein is a powerful modulator of GFR and renal hemodynamics, the mechanisms responsible have not been defined.

Table 2.
EFFECT OF 1 2/3 NEPHRECTOMY
ON RENAL FUNCTION

	SNGFR nl/min	Q_A nl/min	P_{GC} mmHg
Control	27.8	74	49
1 2/3 Nx	62.5	187	63
1 2/3 Nx + low protein diet	28.2	92	46

Hostetter et al. Am J Physiol 241:F85, 1981.

As shown in **Figure 2**, glomerulosclerosis is evident within 7 days following 1-2/3 nephrectomy **(Figure 2, top)**. However, in the rats placed on a low protein diet, glomerulosclerosis is prevented (**Figure 2, bottom**). The correlation between increases in single nephron GFR and glomerular capillary pressure, and sclerosis, led to the description of this condition as "hyperfiltration injury." In addition, 1-2/3 nephrectomy is associated with hypertrophy, which in itself may participate in the development of glomerular and interstitial sclerosis (see below).

A similar sequence of events occurs with bilateral embolization of the kidneys with microspheres (Miller et al, 1990). Following the embolization, ~12% of glomeruli developed ischemic changes. The remaining healthy nephrons hypertrophy and develop sclerosis. This explains how any renal disease which destroys nephrons can lead to hyperfiltration and hypertrophy of the remaining nephrons, leading to their sclerosis and destruction. This may explain why some patients with sickle cell anemia, which usually causes an interstitial renal disease, may develop proteinuria and glomerulosclerosis. The latter lesion is associated with glomerular hypertrophy (Falk et al, 1992).

As indicated in Figure 1, diabetes mellitus is also associated with increases in GFR, glomerular capillary hypertension and hypertrophy. **Figure 3** compares GFR and kidney weight in diabetic (solid circle) and control (open circle) subjects. As can be seen, diabetic patients have larger kidneys with higher GFRs (Puig et al, 1981; Mogensen and Anderson 1973; Christiansen et al 1981; Osterby and Gundersen 1975). The role of these changes in diabetic renal disease have been evaluated in the rat (Zatz et al, 1985). As shown in **Figure 4**, rats made diabetic by an intravenous injection of streptozotocin, develop increases in single nephron glomerular filtration rate, blood flow, and the pressure gradient across the glomerular capillary. It can also be seen in this figure that these variables are also modulated by dietary protein in control and diabetic animals.

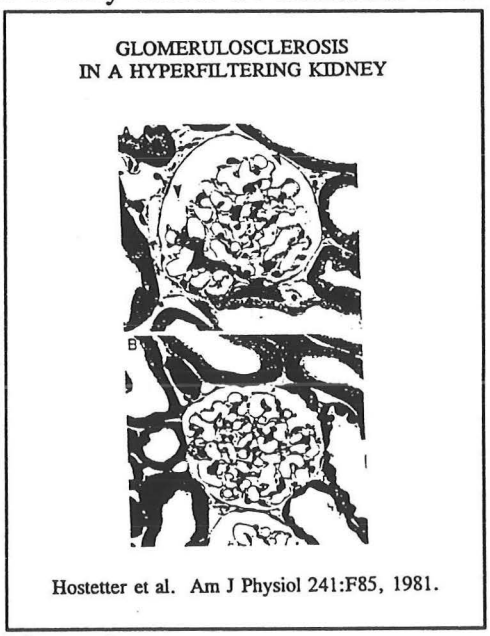
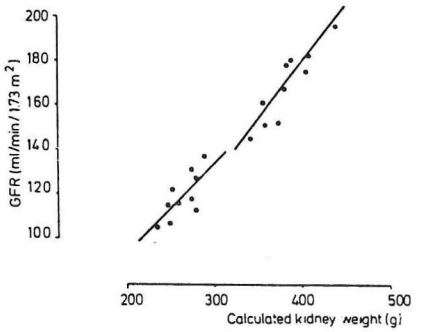


Figure 2

INCREASED GFR AND KIDNEY WEIGHT IN DIABETES MELLITUS



Puig et al. Diabetologia 21:363, 1981.

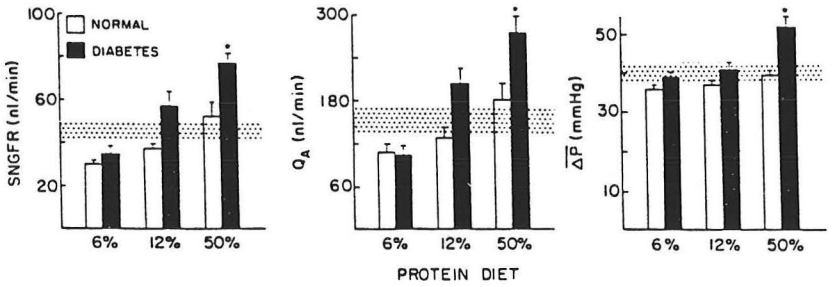
Figure 3

Figure 5 shows albumin excretion as a function of time in control and diabetic animals. As can be seen, there is marked albuminuria in diabetic animals fed a high protein diet. Glomerulosclerosis also was observed almost exclusively in the diabetic animals fed a high protein diet.

A further indication of the importance of hemodynamic changes is evident from case reports of unilateral diabetic glomerulosclerosis (Beroniade et al, 1987; Berkman and Rifkin 1973). In these reports, patients with diabetes mellitus and unilateral renal artery stenosis developed diabetic nephropathy in the kidney with a patent renal artery; the contralateral kidney with the obstructed renal artery was protected.

In none of the above studies is it clear whether the critical lesion is one of hyperfiltration or hypertrophy. Both of these are present in 1-2/3 nephrectomy and diabetes mellitus. Two critical studies have been performed which suggest that hypertrophy is important in the generation of sclerosis. Yoshida et al (1989a) compared three groups of animals. In the first group 2/3 nephrectomy was performed and the

EFFECT OF DIABETES MELLITUS AND DIETARY PROTEIN ON RENAL HEMODYNAMICS



Zatz et al. PNAS 82:5963, 1985.

Figure 4

contralateral kidney remained intact. In the second group, 2/3 nephrectomy was performed and the contralateral kidney was removed. In the third group, 2/3 nephrectomy was performed and the ureter of the contralateral kidney was diverted into the peritoneal cavity. In Figure 6, it can be seen that SNGFR and the glomerular capillary pressure gradient were increased similarly in animals with contralateral nephrectomy and contralateral ureteral diversion at 4 weeks

EFFECT OF DIABETES MELLITUS AND DIETARY PROTEIN ON PROTEINURIA

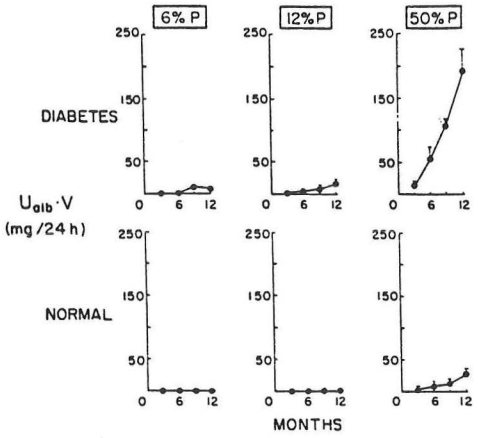
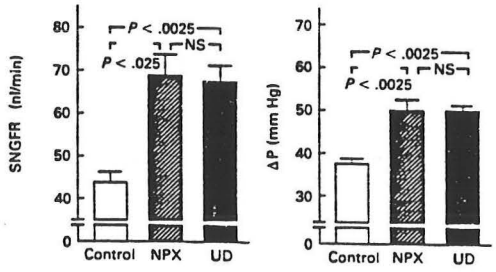


Figure 5

Zatz et al. PNAS 82:5963, 1985.

HYPERTROPHY VS HEMODYNAMICS

Figure 6



Yoshida et al. Kidney Int 35:654, 1989.

HYPERTROPHY VS HEMODYNAMICS

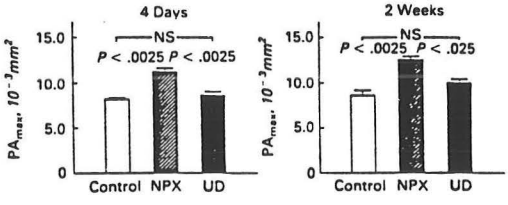


Figure 7

Yoshida et al. Kidney Int 35:654, 1989.

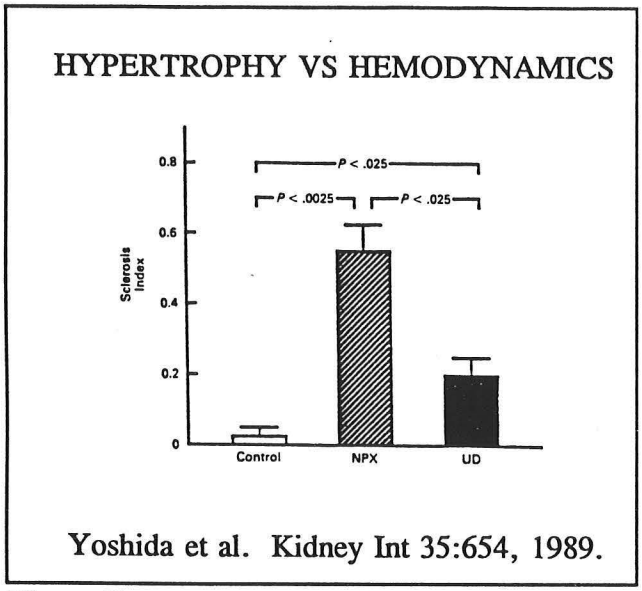


Figure 8

postsurgery. However, as shown in Figure 7, the planar area of glomeruli was increased in animals undergoing contralateral nephrectomy but was not increased in animals undergoing ureteral diversion. This corresponds with measurements of left kidney weight which were increased in the contralateral nephrectomy group at 4 days, 2 weeks, and 4 weeks. Left kidney weight in the contralateral ureteral diversion group was similar to control at 4 days and 2 weeks, and was increased to an intermediate degree at 4 weeks.

These results are of great importance in that they demonstrate that kidney hypertrophy is not merely a consequence of hyperfiltration. With ureteral diversion, the degree of hyperfiltration is identical to that with nephrectomy; but the degree of hypertrophy is far less. It should also be noted that with both nephrectomy and ureteral diversion, the kidney becomes equally nonfunctional. The difference in the level of hypertrophy suggests that the presence of a kidney, even a nonfunctioning one, can suppress growth of the opposite kidney.

Of great significance, Figure 8 shows that the sclerosis index at 4 weeks was greatest in the nephrectomy animals and significantly less in the contralateral ureteral diversion animals. There was some increase in sclerosis index in the contralateral ureteral diversion as compared to control, but this correlated best with hypertrophy. This study suggests that sclerosis correlates better with hypertrophy than with hemodynamic changes.

Table 3.
HYPERTROPHY VS HEMODYNAMICS
1-2/3 NEPHRECTOMY

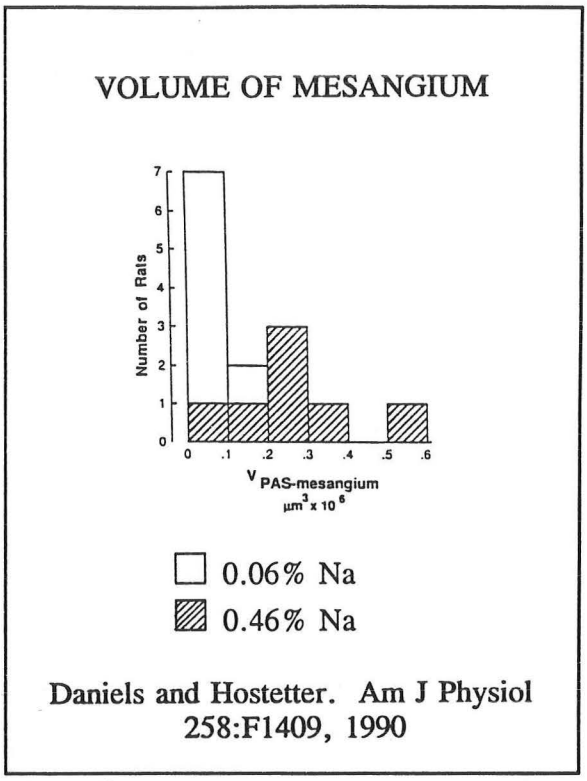
Dietary Na^+, %	SNGFR nl/min	P_{GC} mmHg	Kidney Wt. (g)	Glom Vol $\mu m^3 \times 10^6$	Protein Excretion ng/24 h
0.06	73	65	1.3	1.36	37
0.46	73	67	1.6*	1.84*	81*

Daniels and Hostetter. Am J Physiol 258:F1409, 1990.

Table 3 shows results from a study by Daniels and Hostetter (1990) which examined a similar question. Rats underwent a 1-2/3 nephrectomy, and then were placed on two levels of dietary sodium intake. 0.46% dietary sodium is normal and 0.06% is a very low dietary

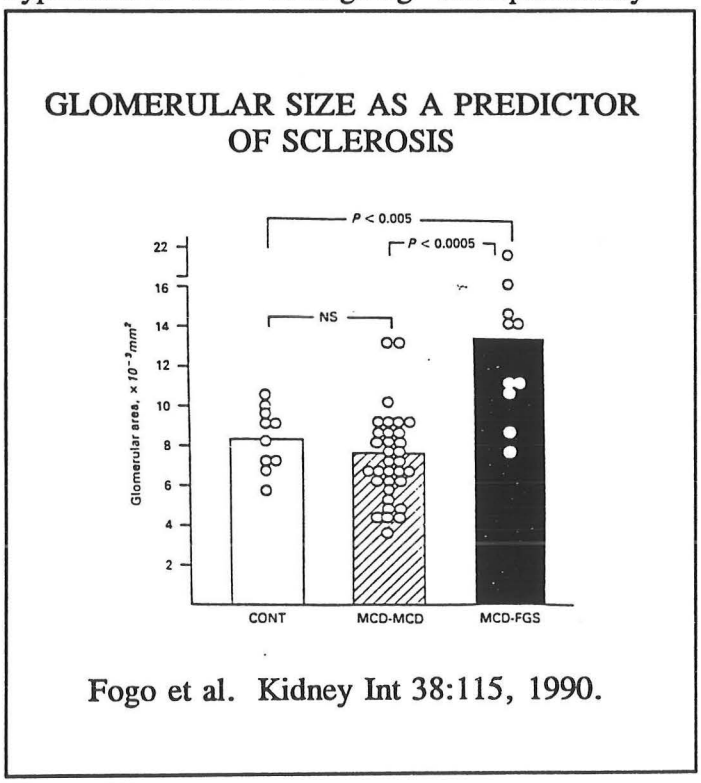
sodium. In both sets of animals SNGFR and glomerular capillary pressure were increased. However, hypertrophy was prevented in the animals on the low salt diet as assessed by kidney weight and glomerular volume. Interestingly, protein excretion was increased in the animals on the normal salt intake but was prevented in the animals on the low salt intake. **Figure 9** shows an assessment of sclerosis. Plotted is the volume of the mesangium, an index of sclerosis. Most of the rats on the low salt diet (open bars) had very low mesangial volumes, whereas rats on the normal salt diet had higher mesangial volumes (striped bars).

A similar effect of dietary salt restriction on progression of renal disease and hypertrophy following 1-2/3 nephrectomy was found by Lax et al (1992). In addition, these investigators found that androgen administration to the low salt animals increased the hypertrophy and the renal damage. Benstein et al (1990) also found that a low salt diet administered to spontaneously hypertensive rats undergoing uninephrectomy prevented hypertrophy and prevented proteinuria and glomerulosclerosis. There was no effect on glomerular capillary pressure or SNGFR.



Daniels and Hostetter. Am J Physiol 258:F1409, 1990

Figure 9



Fogo et al. Kidney Int 38:115, 1990.

Figure 10

A further indication of the importance of hypertrophy is the demonstration of a correlation between glomerular size and the subsequent development of sclerosis in patients with minimal change disease. **Figure 10** shows measurements for renal glomerular area from control patients (clear bar), patients with minimal change disease whose subsequent course was typical for minimal change disease (striped bar; MCD-MCD); and patients with minimal change disease who on subsequent biopsy were found to have focal glomerulosclerosis (MCD-FGS; solid bar) (Fogo et al 1990). As can be seen, in patients with minimal change disease who continue to have minimal change disease, glomerular size is

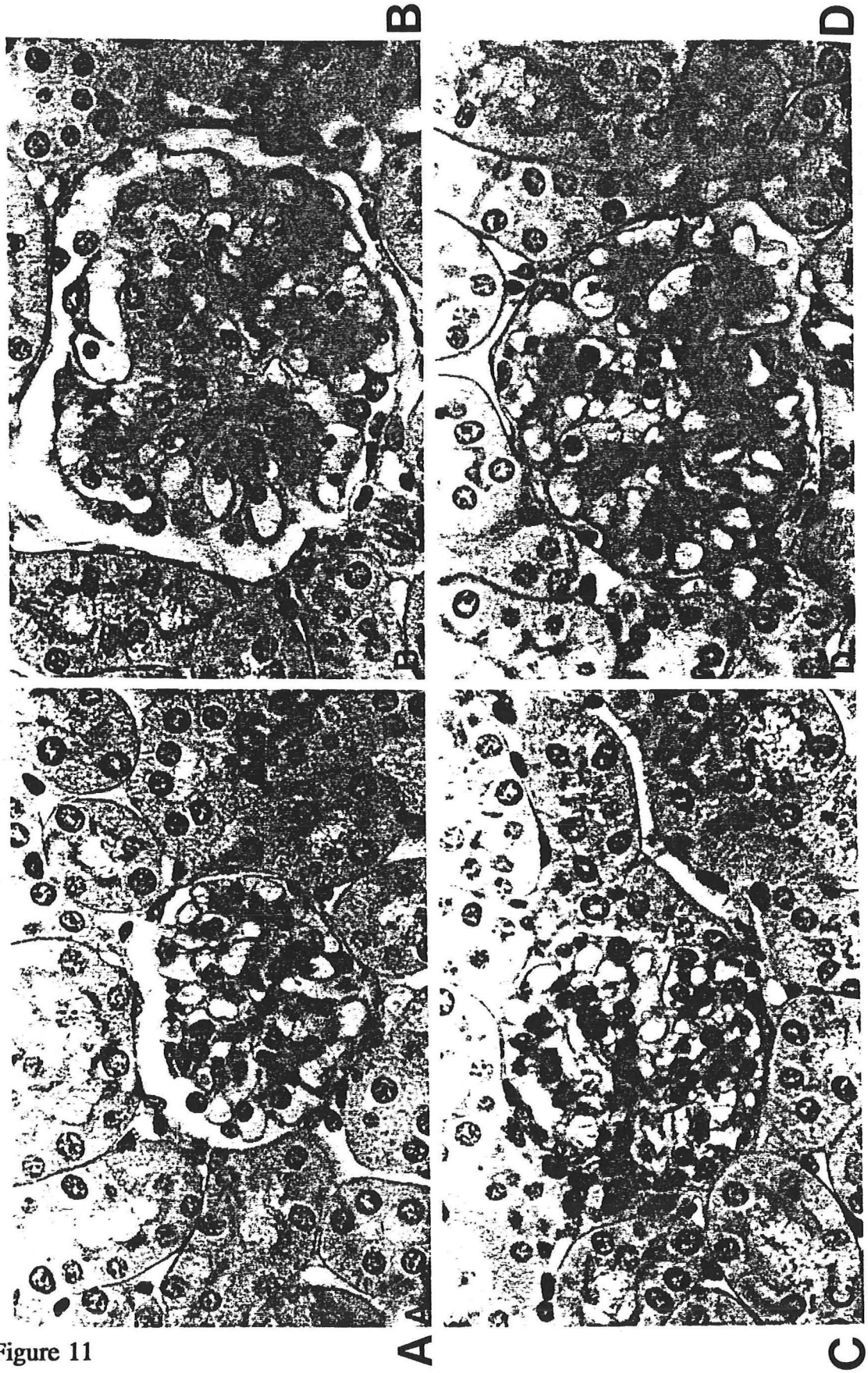


Figure 11

normal. However, patients with minimal change disease who will progress to focal glomerulosclerosis have large glomeruli. This, once again suggests that hypertrophy may be causative in the progression to focal sclerosis. In addition, assessments of glomerular size may be useful in the future as an indicator of which patients with minimal change disease are likely to progress to focal sclerosis.

Perhaps more convincing evidence that hypertrophy can cause renal sclerosis is derived from experiments with animals transgenic for growth factors. Figure 11 shows representative glomeruli from four sets of mice at 14 weeks of age (Doi et al, 1988). "A" shows a glomerulus from a wild type mouse which is essentially normal. In "B", the glomerulus is from a 14 week old mouse transgenic for the growth hormone gene. It can be seen that the glomerulus is increased in size, and in addition there is extensive mesangial proliferation and sclerosis. "C" is a glomerulus from a mouse transgenic for IGF-1 (insulin-like growth factor-1). This glomerulus is also enlarged, but interestingly is not sclerotic. Glomerulosclerosis does not develop in these animals. The reason for the difference between growth hormone and IGF-1 is not presently clear. In "D", a glomerulus is shown from a mouse transgenic for growth hormone releasing factor. Once again, the glomerulus is enlarged and sclerotic.

Similar studies have also been performed in animals transgenic for SV40 large T antigen. In these studies, a metallothionine promoter was used which directs expression in renal tubules (MacKay et al, 1987). SV40 large T antigen is known to increase growth in cells. Transgenic animals developed increased proteinuria at 15 weeks of age as compared to controls. In addition, there was significant glomerulosclerosis detectable as early as 4 weeks of age. Thus, in animals where renal growth is induced by introduction of genes for growth hormone, growth hormone releasing factor, or SV40 large T antigen, rather than secondary to hemodynamic changes, glomerulosclerosis is induced. These studies once again serve to emphasize the importance of growth in the generation of renal sclerosis.

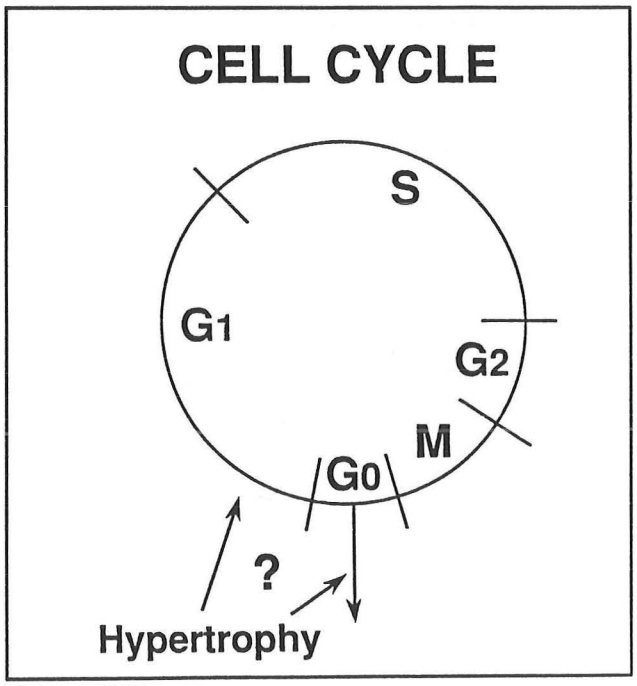


Figure 12

CELLULAR MECHANISMS OF HYPERTROPHY

Figure 12 shows the cell cycle. This cycle describes the multiple stages of cell division or hyperplasia. Resting cells are in G0. Following a stimulus to growth, cells move into a growth phase called G1. In S phase cells replicate their DNA and then enter a second shorter growth phase, G2. Following this, mitosis occurs, and the cell divides into two cells (M phase), both of which then move into the resting G0 state. Our understanding of the cell cycle is based on an enormous amount of research aimed at understanding cell division. This field is of interest in and of itself, but also is of importance in cancer research.

Relatively little work has been done examining the cellular mechanisms responsible for hypertrophy. Based on our

understanding of hypertrophy, cells would not move into the S, G2 or M phase. One issue is whether hypertrophy involves cells moving from G0 to G1 and then aborting their movement through the cell cycle, or whether hypertrophy involves a completely different pathway.

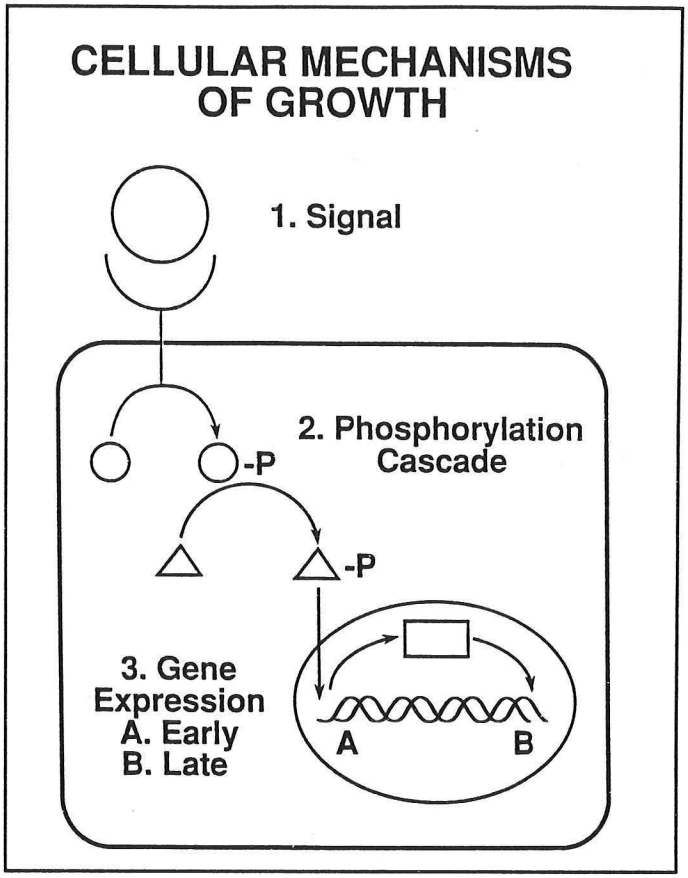


Figure 13

Figure 13 shows the cellular mechanisms responsible for cell division or hyperplasia. For convenience we have divided up the process into three steps. The first step involves an extracellular signal which may be a growth factor interacting with its receptor or some other stimulus to growth. This then leads to activation of signal transduction mechanisms resulting in a phosphorylation cascade. In this cascade, inactive kinases are phosphorylated resulting in active kinases. These kinases then phosphorylate other proteins including kinases. Eventually a large number of proteins in the cell are activated. Some of these then move into the nucleus where they regulate gene expression. Genes which are activated in cell growth have been divided into two categories, immediate early genes or early response genes, and late genes. An immediate early gene is defined as a gene which is activated early following a stimulus to growth, and whose activation is independent of protein synthesis. Thus, if protein synthesis is inhibited by treating cells with cycloheximide, growth factor application will still activate these genes. Late genes, on the other hand, are activated at a later time following cell activation, and require the synthesis of protein intermediates for their activation.

Heart

The cellular mechanisms of hypertrophy have been best studied in the cardiac myocyte. We will therefore discuss results in this system extensively and then examine how they apply to kidney cells. Regarding the first step, cell signalling to cardiac hypertrophy, cardiac myocytes hypertrophy in response to a number of stimuli. When myocytes are examined in culture, they undergo hypertrophy in response to norepinephrine acting through $\alpha 1$ adrenergic receptors (Simpson, 1985; Meidell et al, 1986; Ikeda et al, 1991), angiotensin II (Baker and Aceto, 1990; Aceto and Baker, 1990), endothelin (Ito et al, 1991; Shubeita et al, 1990) or fibroblast growth factors (Parker et al, 1990a; Parker et al, 1990b). While it is possible that these hormones may mediate the effects of increased afterload on the heart, direct effects of stretching myocytes (Komuro et al, 1990; Sadoshima et al, 1992), or of contraction (McDermott and Morgan, 1989) have been demonstrated.

PHOSPHORYLATION CASCADE IN CELL GROWTH

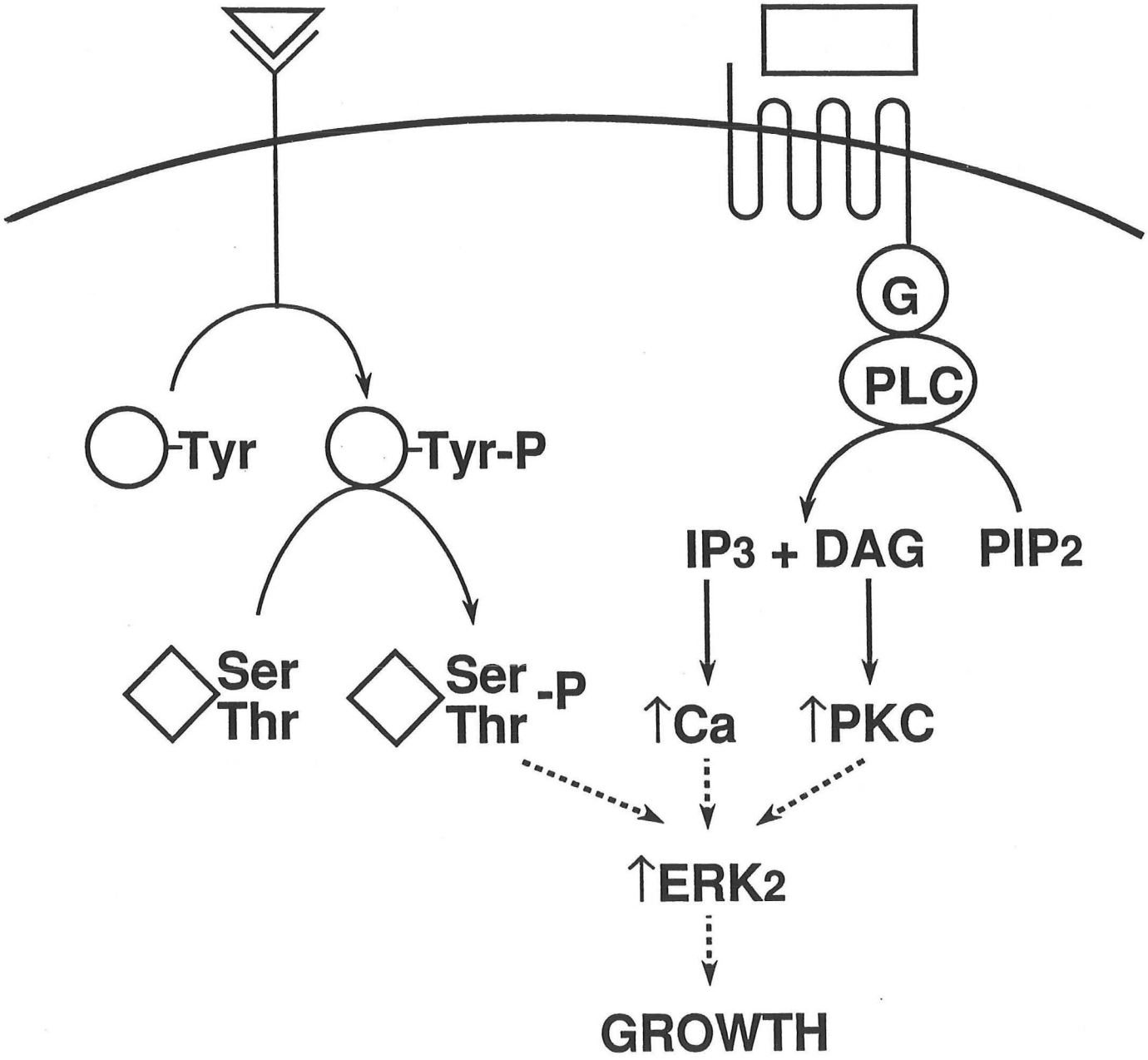
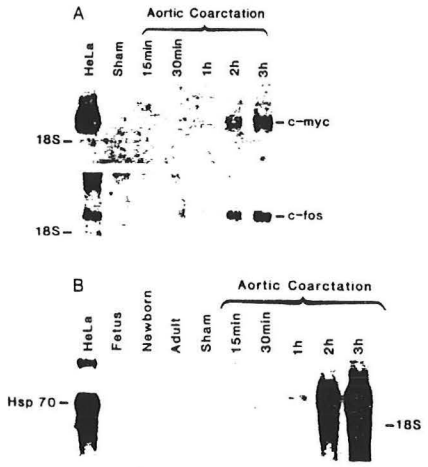


Figure 14

ABDOMINAL AORTIC COARCTATION ACTIVATES EARLY RESPONSE GENES



Izumo et al. PNAS 85:339, 1988.

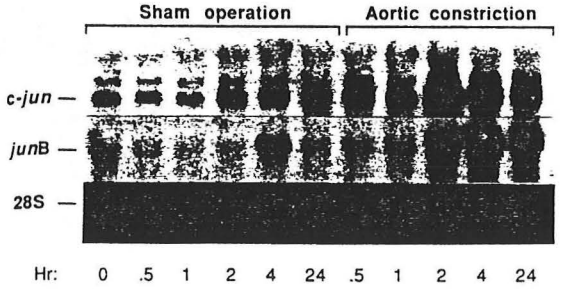
Figure 15a

Figure 14 shows a complex, yet oversimplified, description of the phosphorylation cascade in cell growth (hyperplasia). In general, most agents which stimulate cell growth appear to work through one of two pathways. Shown on the left is the tyrosine kinase pathway. Interaction of a hormone or growth factor with its receptor leads to activation of tyrosine kinase activity in the cytoplasmic domain of the receptor. This leads to autophosphorylation of the receptor and to tyrosine phosphorylation of other proteins, activating a cascade of tyrosine kinases which eventually phosphorylate serine/threonine kinases. One of these serine/threonine kinases phosphorylates the protein ERK-2 (extracellular signal related kinase-2). ERK-2 then phosphorylates a series of substrates eventually leading to cell growth.

An alternative mechanism for activation of growth is for a hormone to react with a G protein-coupled receptor, activating phospholipase C. Phospholipase C causes phosphatidylinositol bisphosphate to be converted to inositol trisphosphate (IP_3) and diacylglycerol (DAG). Inositol trisphosphate causes release of calcium from intracellular stores and diacylglycerol activates protein kinase C. In some manner these pathways are then able to once again phosphorylate and activate ERK-2. In all likelihood additional pathways exist by which ERK-2 can be activated, resulting in cell growth.

Studies in cardiac hypertrophy have suggested an important role for the phospholipase C/protein kinase C pathway (Chien et al, 1991). Angiotensin II causes an increase in cell Ca in cultured chick heart cells (Baker and Aceto, 1990). Endothelin increases IP_3 and DAG production in cultured neonatal rat myocytes (Ito et al, 1991; Shubeita et al, 1990), and increases phosphoinositide turnover in cultured adult rat myocytes (Hilal-Dandan et al, 1992). Endothelin also inhibits adenylyl cyclase in the adult cells (Hilal-Dandan et al, 1992). Increased phosphoinositide turnover is pertussis toxin-insensitive, while inhibition of adenyl cyclase is pertussis toxin-sensitive (implying mediation by a pertussis toxin-sensitive G protein). Norepinephrine causes a shift of protein kinase C from the cytosolic to the membrane fraction (an index of activation) in neonatal rat myocytes (Henrich and Simpson,

ABDOMINAL AORTIC COARCTATION ACTIVATES EARLY RESPONSE GENES



Black et al. J Clin Invest 88:1581, 1991.

Figure 15b

1988). Lastly, activation of protein kinase C with phorbol esters in neonatal rat myocytes leads to hypertrophy, increases in c-fos, c-jun, and egr mRNA, and increases in myosin light chain 2 (Dunnmon et al, 1990). All of these changes occur in hypertrophied myocytes (see below). These results suggest, but still do not prove, an important role for phospholipase C/protein kinase C in myocyte hypertrophy. The role of adenylyl cyclase inhibition is unclear. There is no data on tyrosine kinase pathways in hypertrophy.

A large amount of work has been done in examining immediate early response gene activation in hypertrophy. As described above, these genes are activated early in dividing cells, and their activation is independent of protein synthesis. These genes include c-fos, c-myc, c-jun, junB, erg-1, and many others. Figure 15 shows that suprarenal aortic coarctation causes increases in mRNAs for c-myc, c-fos, HSP-70 (Izumo et al, 1988), c-jun, and junB (Black et al, 1991).

As noted above, it is not presently clear whether the hypertrophy which occurs in response to coarctation is mediated by stretch itself or by a number of hormones. Stretching cultured cardiac myocytes leads to increases in mRNA for c-fos, c-jun, egr-1, and c-myc (Komuro et al, 1990; Sadoshima et al, 1992). Similarly, treatment of cultured neonatal rat cardiac myocytes with α-adrenergic agonists leads to increases in c-fos, c-jun, and egr-1 (Iwaki et al, 1990). Endothelin also activates c-fos and egr-1 in these cells (Shubeita et al, 1990).

In addition to activation of early response genes, cardiac hypertrophy is associated with activation of genes more related to cardiac structure and function. Figure 16 shows cardiac myocytes stained with antisera against myosin light chain 2 (Chien et al, 1991). On the left are control myocardial cells, while on the right are cells treated with phenylephrine, an α adrenergic agonist. This increase in myosin light chain 2 is associated with increases in the mRNA for this species.

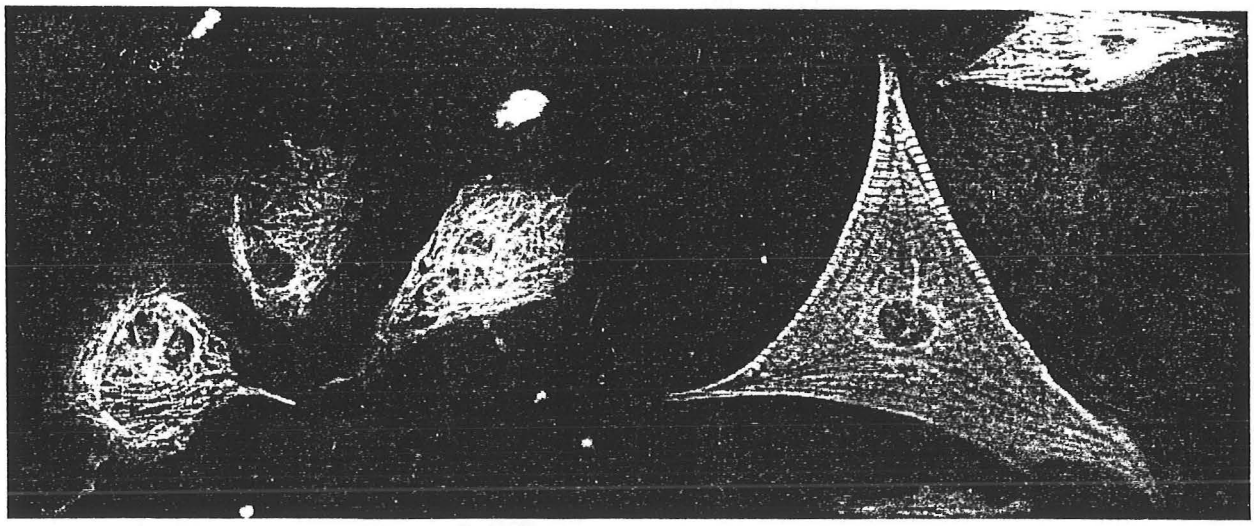


Figure 16

Figure 17 shows effects of suprarenal aortic coarctation on myosin heavy chain mRNA (Izumo et al, 1987). Myosin heavy chain exists as two isoforms, an alpha and a beta isoform. In the rat, the beta isoform dominates in the fetus, while the alpha isoform dominates in adults. Sham operation in adults has no effect, leaving the alpha isoform as the dominant species. However, following coarctation of the aorta there is an increase in beta myosin heavy chain

mRNA seen at 4, 6, and 15 days. Thus, the hypertrophic process is associated with the expression of the fetal isoform. Interestingly, thyroid hormone which also causes hypertrophy, does not lead to expression of the beta isoform but rather increases expression of the alpha isoform. When animals with coarctation are treated with thyroid hormone, expression of the beta isoform is actually suppressed by thyroid hormone. A similar isoform switch has been found with α_1-adrenergic receptor stimulated hypertrophy in cultured myocytes (Waspe et al, 1990). This isoform switch may be important in that the alpha isoform has a higher ATPase activity and is associated with more rapid contraction, while the beta isoform has lower ATPase activity and contracts more slowly. Thus, the switch from alpha to beta may be associated with decreased contractility.

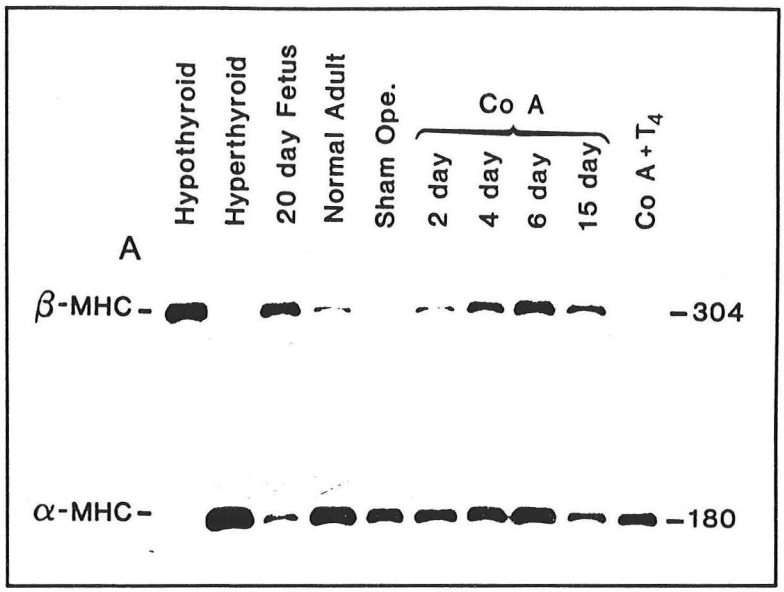
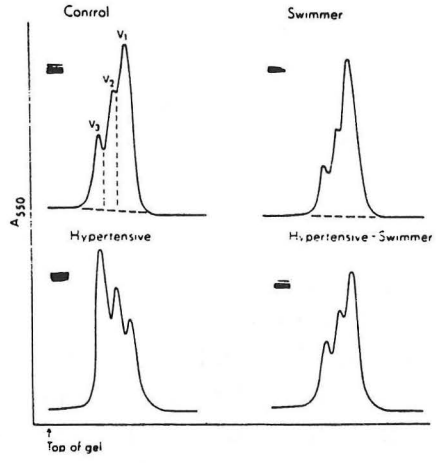


Figure 17

A similar interaction occurs between exercise and hypertension (Scheuer et al, 1982). **Figure 18** shows myosin heavy chain isozyme separation on pyrophosphate gels. The V1 peak represents an alpha-alpha homodimer, the V2 peak an alpha-beta heterodimer, and the V3 peak a beta-beta homodimer. As discussed above, in the adult rat the dominant isoform is alpha, and thus the dominant isozyme is V1. When rats are exercised by swimming, the heart hypertrophies but the pattern of isozymes remains similar to control. However, when rats are made hypertensive with a renal artery clip, there is a shift to the V3 isozyme, consistent with the switch to the beta isoform. If the hypertensive rats are then exercised by swimming, the pattern returns to normal similar to the effect of treating rats with coarctation with thyroid hormone. Thus, exercise and thyroid hormone have similar effects on myosin heavy chain and this effect is very

EFFECT OF EXERCISE AND HYPERTENSION ON MYOSIN HEAVY CHAIN ISOFORMS



Scheuer et al. J Clin Invest 70:1300, 1982.

Figure 18

Table 4.
A CLASSIFICATION OF GENETIC PROGRAMS
DURING MYOCARDIAL CELL HYPERTROPHY

Induction of Immediate Early Genes	Induction of Embryonic Genes	Induction of Constitutive Contractile Protein Genes
c-fos	β MHC	MLC-2
c-jun	ANF	Cardiac α actin
jun-B	Skeletal α actin	
egr-1	Smooth muscle lactin	

Adapted from Chien et al. FASEB J 5:3037, 1991.

different from the effect of an increased afterload.

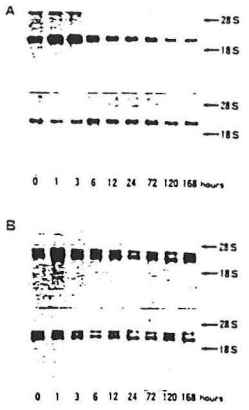
Table 4 summarizes the genetic response during myocardial cell hypertrophy (Chien et al, 1991). A series of immediate early genes are activated such as c-fos, c-jun, junB, and egr-1 (Ito et al, 1991; Shubeita et al, 1990; Komuro et al, 1990; Izumo et al, 1988; Black et al, 1991; Iwaki et al, 1990; Starksen et al, 1986; Schunkert et al, 1991; Dunnmon et al, 1990; Sadoshima et al, 1992). Other genes which are activated can be divided into two categories. In one category, there is induction of genes which are ordinarily expressed constitutively, including myosin light chain 2 and cardiac alpha actin (Shubeita et al, 1990; Ito et al, 1991; Chien et al, 1991; Dunnmon et al, 1990; Knowlton et al, 1991; Lee et al, 1988; Long et al, 1989). On the other hand, a number of fetal genes that normally are not expressed in adult ventricular myocytes are expressed in hypertrophy. These include the beta isoform for myosin heavy chain, skeletal muscle alpha actin, smooth muscle alpha actin, and atrial natriuretic factor (Ito et al, 1991; Shubeita et al, 1990; Izumo et al, 1987; Black et al, 1991; Izumo et al, 1988; Waspe et al, 1990; Bishopric and Kedes, 1991; Knowlton et al, 1991; Bishopric et al, 1987; Long et al, 1989; Sadoshima et al, 1992).

Similar studies are now being performed examining mechanisms of renal tubular hypertrophy. Angiotensin II and increases in glucose concentration have been shown to cause hypertrophy in an SV40 transformed mouse proximal tubule cell line (Ziyadeh et al, 1990; Wolf et al, 1991; Wolf and Neilson, 1990). Insulin and ammonia have been demonstrated to cause hypertrophy in primary cultures of rabbit proximal tubule cells (Fine et al, 1985a; Golchini et al, 1989). Transforming growth factor β has been shown to convert a hyperplastic response to a hypertrophic response in rabbit proximal tubule primary cultures (Fine et al, 1985b). Although the signalling pathways have not been defined completely, angiotensin II has been suggested to work through inhibition of adenylyl cyclase (Wolf et al, 1991). S6 kinase, a substrate of ERK-2 was found to be activated with uninephrectomy by one group (Harris, 1992), but not by another (Alberti et al, 1992).

The role of immediate early response genes in renal hypertrophy has been controversial. When one kidney is removed, and mRNA is measured in the contralateral kidney, some

investigators have found increases in these genes (Nakamura et al, 1992; Sawczuk et al, 1990), while other investigators have found no response (Beer et al, 1987; Kujubu et al, 1991; Ouellette et al, 1990; Norman et al, 1988). The results of Nakamura et al (1992) are shown in **Figure 19** (A: c-fos; B: c-jun).

UNINEPHRECTOMY INCREASES c-fos AND c-jun mRNA IN THE CONTRALATERAL KIDNEY



Nakamura et al. Am J Physiol 262:F389, 1992.

Figure 19

We recently studied the effect of acidosis on early response genes. As noted previously, acidosis causes renal hypertrophy. We examined the effect of incubating an SV40 transformed mouse proximal tubule cell line in acid media (Horie et al, 1992). **Figure 20** shows that acid incubation increased c-fos, c-jun, junB, and egr-1 mRNAs.

In renal tubules, constitutive transport protein genes would be the equivalent of constitutive contractile protein genes in the heart. Sodium and bicarbonate absorption in the proximal tubule are mediated by an apical membrane Na/H antiporter and a basolateral membrane Na/3HCO_3 symporter. Proximal tubular Na/H antiporter activity is increased in uninephrectomy, diabetes, acidosis, and K deficiency, all of which are associated with renal hypertrophy (Preisig and Alpern, 1991; Harris et al, 1984; Preisig and Alpern, 1988; Soleimani et al, 1990; Harris et al, 1986; Akiba et al, 1987). Parallel increases in Na/3HCO_3 symporter activity have been found with uninephrectomy, acidosis, and K deficiency (Preisig and Alpern, 1988; Preisig and Alpern, 1991; Akiba et al, 1987; Soleimani et al, 1990). When proximal tubule cell cultures, or cultures of the above described SV40 transformed proximal tubule cell line are exposed to acid media, Na/H antiporter activity and mRNA (NHE-1 isoform) are increased (Horie et al, 1990; Moe et al, 1991).

Based on the above results, it appears that cell hypertrophy is likely mediated by mechanisms similar to those occurring in hyperplasia. An extracellular signal is perceived, possibly through a mechanical event or possibly through secretion of growth factors and their interaction with receptors. This then activates a series of signalling pathways, which have not been completely defined in hypertrophy. Protein kinase C likely plays a key role, but the role of tyrosine kinases has not been examined. Immediate early response gene activation appears to be similar in hyperplasia and hypertrophy, although this issue is not completely settled in renal hypertrophy. The expression of fetal isoforms may represent a dedifferentiation of the cells as they move into the growth phase. Based on the above similarities, it is likely that in both hypertrophy and hyperplasia, cells move from G0 to G1. The difference between hyperplasia and hypertrophy is that in hypertrophy the cell cycle arrests in G1, while in hyperplasia cells proceed into S, G2, and M. The mechanisms responsible for these differences between hypertrophy and hyperplasia have not been elucidated.

IMPLICATIONS FOR TREATMENT

Based on the previous discussions, a number of agents may be useful in preventing hypertrophy. As shown previously, angiotensin II is a potent growth factor in cardiac myocytes and in renal tubuloepithelial cells. It is also a potent growth factor in vascular smooth muscle cells. In all these cells hypertrophy results. Thus, agents which block angiotensin II may, in addition to other effects, prevent hypertrophy. Such agents may work as angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, or renin inhibitors. Thus far, there has been extensive clinical experience with converting enzyme inhibitors only. Calcium channel blockers may be important in situations where calcium entry into the cell through voltage sensitive L-type channels is key to the hypertrophic response. Low dietary protein and salt restriction may be important in preventing renal hypertrophy. Lastly, endothelin has been shown to be a potent growth factor in cardiac myocytes and in renal cells. Receptor blockers are presently being developed for endothelin. In addition, active endothelin is formed from big endothelin through proteolytic cleavage by a converting enzyme. Thus, the potential for converting enzyme inhibitors exists.

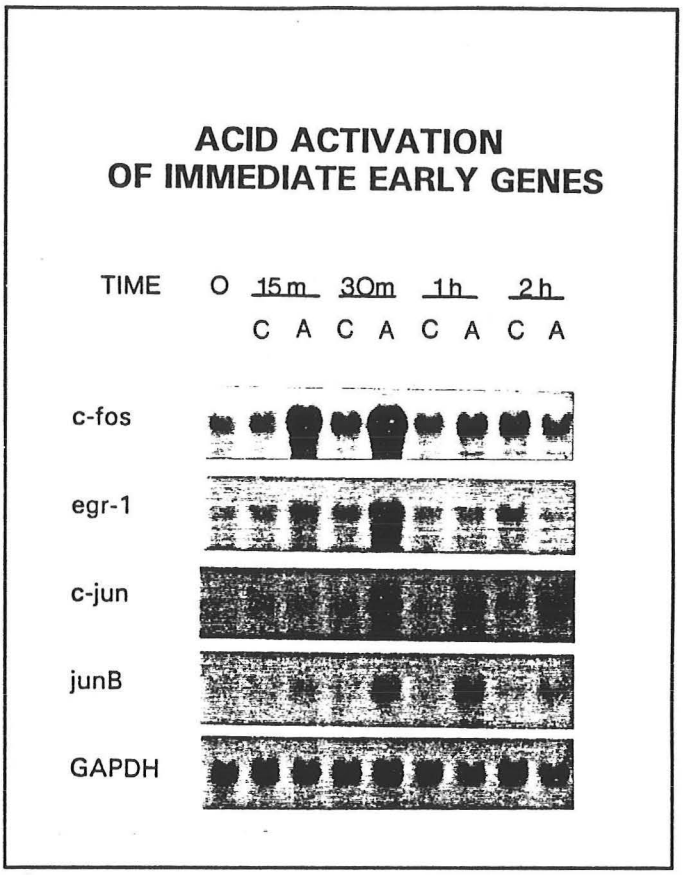


Figure 20

Angiotensin II: Converting Enzyme Inhibitors

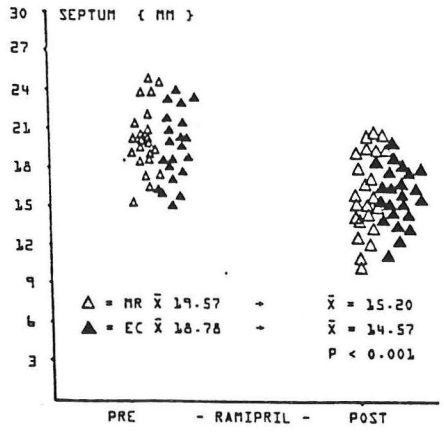
As noted above, angiotensin II is a potent growth factor in cardiac myocytes, vascular smooth muscle cells, and the kidney. Thus, in many respects it holds great potential for being a clinically important growth factor. In addition, the safety and potency of converting enzyme inhibitors allow inhibition of this system. For this reason, there have been a number of trials examining the effects of converting enzyme inhibitors.

It is important to realize that angiotensin II may be an important growth factor even in conditions where circulating angiotensin II levels are low. This is because numerous tissues have been demonstrated to possess local renin angiotensin systems. Thus, while the majority of circulating renin is produced in the kidney, many different tissues can produce renin, angiotensin II, and converting enzyme, enabling them to produce angiotensin II.

Heart

Extensive studies have been performed demonstrating the usefulness of converting

REGRESSION OF CARDIAC HYPERTROPHY IN HYPERTENSIVE PATIENTS TREATED WITH RAMIPRIL X 3 MONTHS
(MR = magnetic resonance;
EC = echocardiography)



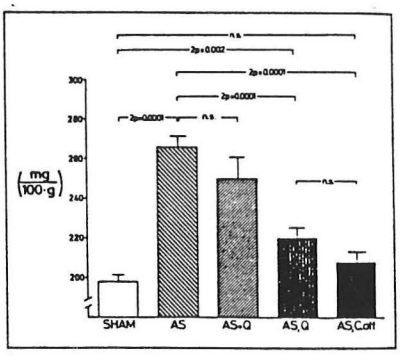
Eichstädt et al. Am J Cardiol 59:98D, 1987.

Figure 21

enzyme inhibitors in treating myocardial hypertrophy. **Figure 21** shows results of studies where patients were treated for three months with ramipril (Eichstädt et al, 1987). Left ventricular septal wall thickness was measured either by magnetic resonance tomography (open triangles) or echocardiography (closed triangles). As can be seen, three months of treatment with ramipril decreased septal thickness. These results could be due to inhibition of local angiotensin II generation with inhibition of cell growth, or could be secondary to decreases in blood pressure and cardiac afterload.

To examine this further, studies have examined the effect of another converting enzyme inhibitor, quinapril, on cardiac mass in rats with a model of aortic stenosis, stenosis of the ascending aorta induced with a clip (Kromer and Riegger, 1988). **Figure 22** shows results. Compared to sham operated animals, six weeks of aortic stenosis (AS) caused a large increase in left ventricular mass. Treatment with quinapril during the six weeks (AS+Q) did not prevent cardiac hypertrophy. However, as noted in the bar on the right (AS, Coff), following six weeks of aortic stenosis, if the clip was removed for six weeks, cardiac hypertrophy regressed. If, on the other hand, aortic stenosis was present for 12 weeks and quinapril was added for the last six weeks (AS,Q), a similar regression of cardiac hypertrophy was induced. In this case, quinapril treatment was equivalent to removing the clip. These results cannot be explained by decreases in afterload, as the afterload is due to a fixed obstruction. This suggests that regression of cardiac hypertrophy by converting enzyme inhibitors is secondary to effects on growth.

REGRESSION OF CARDIAC HYPERTROPHY IN "AORTIC STENOSIS" WITH QUINAPRIL



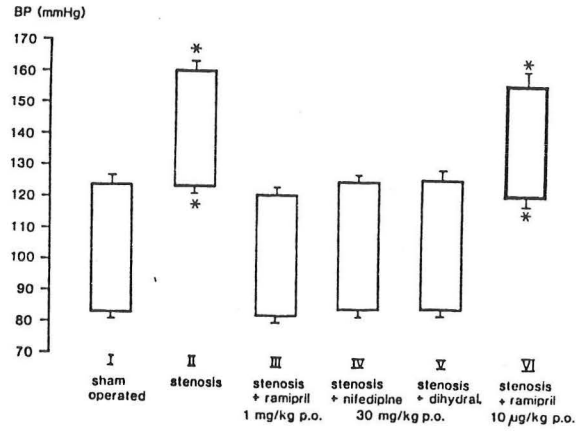
Kromer and Riegger. Am J Cardiol 62:161, 1988.

Figure 22

Linz et al (1989) examined the effect of antihypertensive agents on left ventricular mass in rats made

hypertensive by suprarenal aortic ligation. As shown in Figure 23a, stenosis of the aorta caused hypertension. This was treated to a similar extent with ramipril, nifedipine, or dihydralazine. A low dose of ramipril was not able to control blood pressure. When they examined left ventricular weight (Figure 23b), stenosis caused an increase in left ventricular weight after six weeks. In spite of similar levels of blood pressure control by ramipril, nifedipine, and dihydralazine, only ramipril was able to decrease left ventricular weight to that of sham operated animals. In addition, low doses of ramipril that had no effect on blood pressure, caused a decrease in left ventricular weight. Once again, these studies are best explained by a specific growth effect of angiotensin II and anti-growth effect of converting enzyme inhibitors.

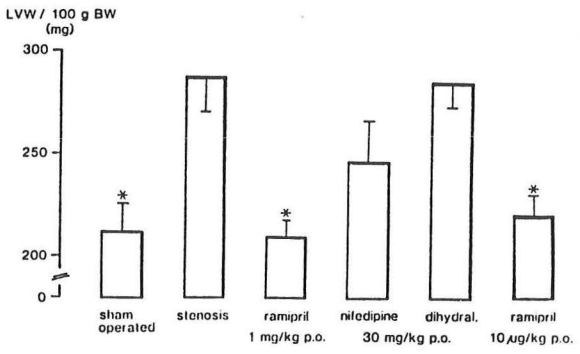
REGRESSION OF CARDIAC HYPERTROPHY WITH RAMIPRIL IN THE HYPERTENSIVE RAT
(suprarenal aortic ligation)



Linz et al. Clin Exper Hyper-Theory and Practice A11(7):1325, 1989.

Figure 23a

REGRESSION OF CARDIAC HYPERTROPHY WITH RAMIPRIL IN THE HYPERTENSIVE RAT
(suprarenal aortic ligation)



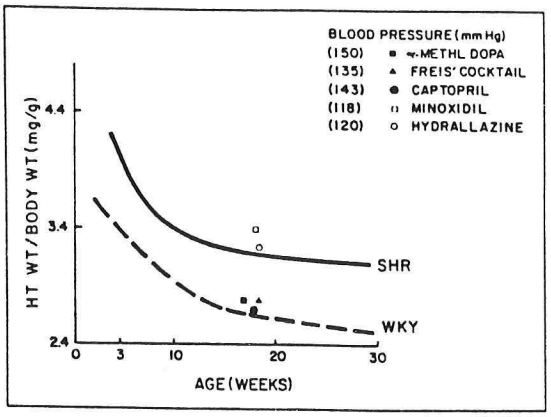
Linz et al. Clin Exper Hyper-Theory and Practice A11(7):1325, 1989.

Figure 23b

Additional supportive studies have been performed in spontaneously hypertensive rats (SHR). This inbred strain of rats develops severe hypertension. Wistar-Kyoto rats (WKY) are normotensive inbred rats from which the spontaneously hypertensive rats have been derived. Figure 24 shows effects of blood pressure treatment in SHR rats on heart weight normalized for body weight (Sen, 1989). As can be seen, in normal rats heart weight divided by body weight decreases as the rats grow. However, at all ages SHR rats have a greater heart weight per body weight compared to WKY rats. Interestingly, if SHR rats are treated with hydralazine, cardiac weight is unaffected, in spite of good blood pressure control. Treatment of the hypertension with minoxidil actually increases cardiac size, in spite of good blood pressure

control. On the other hand, treatment of hypertension with alpha methyldopa or Freis' cocktail (a mixture of antihypertensives) decreases cardiac mass. The most effective agent at decreasing cardiac mass in these studies was captopril. In fact, captopril decreased cardiac mass in SHR rats to that of WKY rats. These studies once again suggest a specific effect of converting enzyme inhibitors on growth. Afterload reduction in these studies should be as great with minoxidil or hydralazine as with captopril; yet only captopril decreased cardiac mass.

EFFECT OF ANTIHYPERTENSIVE THERAPY ON HEART WEIGHT IN THE SPONTANEOUSLY HYPERTENSIVE RAT



Sen. Am J Med 9/26/89, p. 87

Figure 24

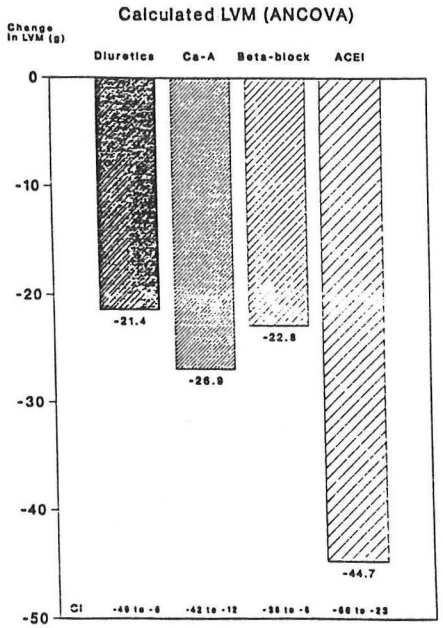
Support for these rat studies is derived from a recently published metaanalysis of 109 treatment studies examining the effect of antihypertensives on reversal of left ventricular hypertrophy (Dahlof et al, 1992). Left ventricular hypertrophy was assessed by echocardiography in all studies. **Figure 25** shows that converting enzyme inhibitors were superior to diuretics, calcium channel blockers, and β-blockers.

There is also additional support for a beneficial effect of converting enzyme inhibitors in patients with heart failure. A number of large trials have been performed showing beneficial effects of these agents. Here we shall review a few of the most important trials. The CONCENSUS trial (Cooperative North Scandinavian Enalapril Survival Study, 1987) examined the effect of enalapril versus placebo in patients with New York Heart Association Class IV congestive heart failure. As shown in **Figure 26**, patients treated with enalapril had improved survival. In addition, the patients treated with enalapril more frequently shifted to better NYHA classes. Out of 127 patients treated with enalapril, 3 patients became Class I, 13 patients became Class II, and 38 patients became Class III.

The SOLVD Study (Studies of Left Ventricular Dysfunction, 1991) examined patients with cardiac ejection fractions less than 35%. Ninety percent of these patients were New York Heart Association Classes II and III. Once again, enalapril was compared to placebo. **Figure 27** shows that there was again improved mortality in the enalapril-treated patients. Most of this improvement was related to death from progressive heart failure. There was no effect on death from arrhythmias. In addition, as shown in Figure 28 there was a decreased percentage of events, defined as death or hospitalization for congestive heart failure in the enalapril group.

In both of the above studies, enalapril was compared to placebo, and it is not clear whether the effect would be found with any vasodilator. The V-HeFT II (Veterans Administration Cooperative Vasodilator-Heart Failure Trial) examined patients with ejection fractions less than 45%, and compared enalapril with a combination of hydralazine and isosorbide dinitrate (Cohn et al, 1991). Cumulative mortality was lower in the enalapril group

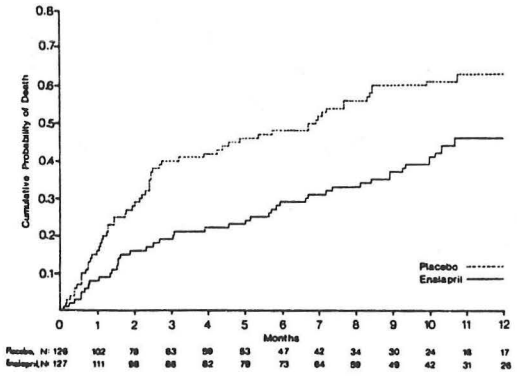
DECREASE IN LEFT VENTRICULAR MASS
WITH ANTIHYPERTENSIVE TREATMENT
A Metaanalysis of 109 Studies



Dahlöf et al. Amer J Hypertens 5:95, 1992.

Figure 25

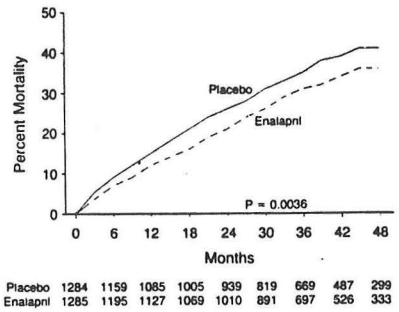
CONSENSUS TRIAL
Cooperative North Scandinavian
Enalapril Survival Study
(NYHA Class IV)



N Engl J Med 316:1429, 1987.

Figure 26

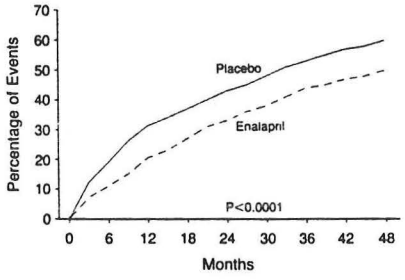
SOLVD
Studies of Left Ventricular Dysfunction
(EF<35%; 90% NYHA II + III)



N Engl J Med 325:293, 1991.

Figure 27

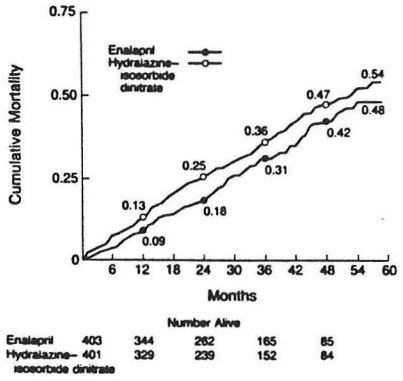
SOLVD
Studies of Left Ventricular Dysfunction
(EF<35%; 90% NYHA II + III)



N Engl J Med 325:293, 1991.

Figure 28

V-HeFT II
Veterans Administration Cooperative
Vasodilator-Heart Failure Trial
(EF < 45%)



Cohn et al. N Engl J Med 325:303, 1991.

Figure 29

Table 5.
ANNUAL MORTALITY RATE
V-HeFT II

NYHA Class	Enalapril	Hydralazine/ Isosorbide Dinitrate
I	8.2	14.9
II	8.2	11.9
III + IV	16.5	16.8

(**Figure 29**). In this study, there was a difference in the rate of sudden death. **Table 5** shows the mortality rate with enalapril versus hydralazine-isosorbide dinitrate in the different New York Heart Association classes. It can be seen that the greatest benefit was observed in patients with Classes I and II heart failure. It is important to note that in the V-HeFT I trial hydralazine-isosorbide dinitrate was shown to be better than placebo (Cohn et al, 1986). Patients treated with hydralazine-isosorbide dinitrate did equally well in the V-HeFT I and II trials.

Kidney

Extensive studies have been performed demonstrating the beneficial effect of converting enzyme inhibitors in slowing the progression of renal disease in rats with reduced renal mass. The first study in this regard was performed by Anderson et al (1985), and results are shown in **Table 6** and **Figure 30**. As can be seen, in rats with 1-2/3 nephrectomy, enalapril lowered

pressure in the glomerular capillary without significantly affecting single nephron GFR. As shown in the figure, after 8 weeks untreated rats had larger glomeruli with mesangial expansion (top), while treated rats had smaller, normal appearing glomeruli (bottom). Enalapril treated

Table 6.
ENALAPRIL PREVENTS PROGRESSION
OF RENAL DISEASE IN RATS
WITH REDUCED RENAL MASS

	SNGFR nl/min	P_{GC}
1-2/3 Nx	93	69
1-2/3 Nx + Enalapril	82	54
	NS	$p<0.001$

Anderson et al. J Clin Invest 76:612, 1985.

rats also had a decrease in the level of proteinuria as compared to controls (Figure 31). The finding that enalapril decreases sclerosis and proteinuria along with glomerular capillary pressure, while at the same time not significantly affecting single nephron GFR, has been interpreted to indicate that the key variable is glomerular capillary pressure rather than SNGFR. It should be noted that the correlation between glomerular capillary pressure and renal disease does not prove cause-effect. Indeed, enalapril also prevents hypertrophy.

While there is little question that enalapril is beneficial in the progression of renal disease in rats, controversy exists as to whether other antihypertensive regimens are equally effective. Some investigators have found that any antihypertensive regimen that lowers blood pressure to the same extent as enalapril is equally effective in slowing the progression of renal disease (Yoshida et al, 1989b), while others have found that only enalapril is effective (Anderson et al, 1986).

In a recently published open study, patients with GFRs varying from 6-54 ml/min/1.73 m^2 were randomized to enalapril or conventional antihypertensive treatment (Kamper et al, 1992). Patients were followed for at least 2 years, and GFR was assessed as the clearance of ^{51}Cr-EDTA. The rate of decrease in GFR decreased from -0.31 in the control group to -0.20 ml/min/1.75 m^2/month in the enalapril group.

Enalapril has also been demonstrated to slow progressive of renal disease in rats with diabetes mellitus. **Figure 32** shows the effect of enalapril on glomerular hemodynamics in these rats (Zatz et al, 1986). Diabetic rats have an increased single nephron GFR and plasma flow, and glomerular capillary pressure gradient. Once again, enalapril does not return single nephron GFR or plasma flow to normal, but does decrease the glomerular capillary pressure gradient to normal. **Figure 33** shows that diabetes causes increased proteinuria, and that this is prevented by enalapril.

An open study has also been performed comparing enalapril with metoprolol in patients with diabetic nephropathy (Björck, 1992). The rate at which GFR decreased per year was

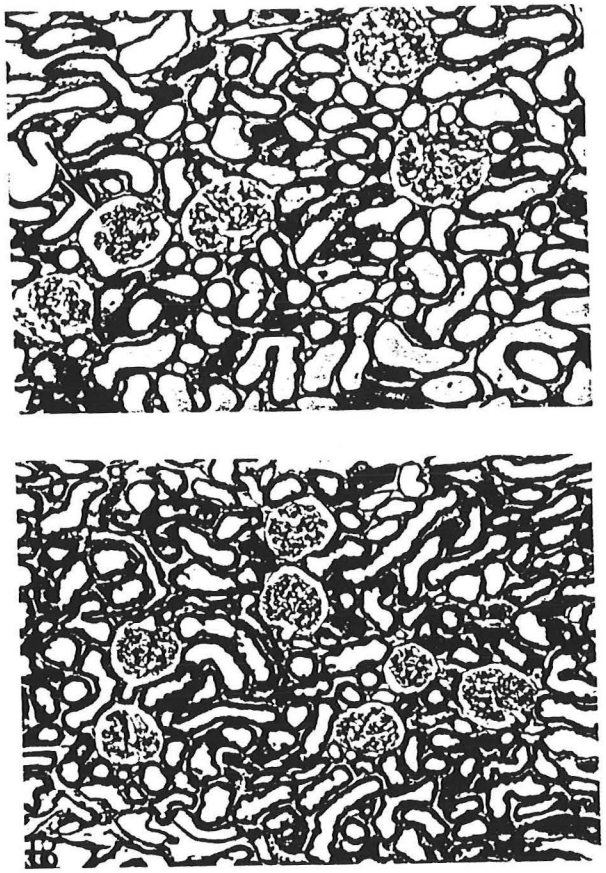
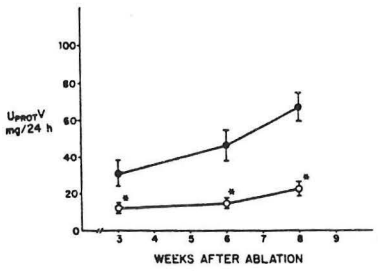


Figure 30

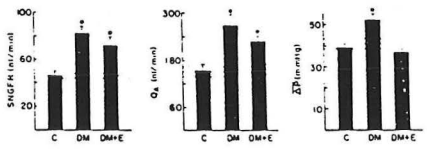
ENALAPRIL PREVENTS PROGRESSION
OF RENAL DISEASE IN RATS
WITH REDUCED RENAL MASS
(● Control, ○ Enalapril)



Anderson et al. J Clin Invest 76:612, 1985.

Figure 31

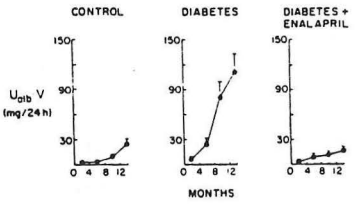
ENALAPRIL PREVENTS PROGRESSION
OF RENAL DISEASE IN RATS
WITH DIABETES MELLITUS



Zatz et al. J Clin Invest 77:1925, 1986.

Figure 32

ENALAPRIL PREVENTS PROGRESSION
OF RENAL DISEASE IN RATS
WITH DIABETES MELLITUS



Zatz et al. J Clin Invest 77:1925, 1986.

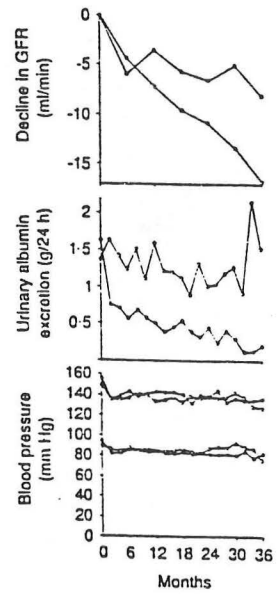
Figure 33

significantly slowed and the amount of proteinuria decreased in human subjects treated with enalapril, in spite of similar levels of hemoglobin A1C and similar degrees of blood pressure control (Figure 34).

While slowing the progression of diabetic nephropathy is a laudable goal, an even more important goal is the prevention of diabetic nephropathy. Mathiesen et al (1991) performed an open, prospective, randomized trial on 44 normotensive patients with insulin dependent diabetes mellitus and microalbuminuria, comparing captopril to placebo. Figure 35 shows that captopril decreased proteinuria, in the absence of an effect on blood pressure. In addition, over the course of follow-up seven control patients developed overt diabetic nephropathy while none of the treated patients did.

In summary, converting enzyme inhibitors appear to be promising agents in the treatment of patients with heart disease, kidney disease, and hypertension. Data clearly show effects which cannot be attributed to measurable hemodynamic changes. At least a part of these beneficial effects is likely attributable to the ability of converting enzyme inhibitors to prevent angiotensin II-induced cell growth.

ENALAPRIL VS METOPROLOL
IN DIABETIC NEPHROPATHY



Björck et al. BMJ 304:339, 1992.

Figure 34

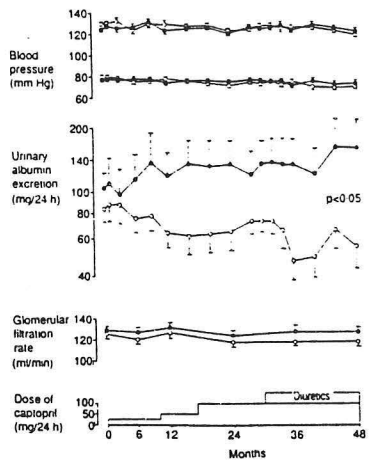
Calcium Channel Blockers

A number of studies have examined the effect of calcium channel blockers in modulating the progression of renal disease. In general, many of these studies in rats have been negative (Pelayo et al, 1988; Brunner et al, 1989; Wight et al, 1990), suggesting that calcium channel blockers are not as effective as converting enzyme inhibitors. However, Harris et al (1987) found that verapamil prevented renal damage in the 1-2/3 nephrectomy model, in spite of no effect on blood pressure. Dworkin et al (1990) have shown that in the model of DOCA-salt hypertension in rats, nifedipine is more effective than enalapril. Nifedipine decreased blood pressure and inhibited the development of proteinuria in this model. Of note, nifedipine did not affect glomerular capillary pressure, but decreased glomerular volume and inhibited the development of glomerulosclerosis. These studies thus, similar to those described previously, dissociate progression of renal disease from glomerular capillary pressure. Interestingly, in this model of volume-dependent hypertension, enalapril did not affect blood pressure, urinary protein excretion, kidney weight, or GFR. In this model, control of blood pressure with hydrochlorthiazide, hydralazine, and reserpine lowered blood pressure, but did not protect the kidney (Dworkin et al, 1987).

In agreement with these results, Nickerson (1984) examined the effect of nitrendipine, another calcium channel blocker, on kidney and heart pathology in DOCA-salt hypertension. A low dose of nitrendipine was used which did not lower systolic blood pressure. In spite of this, kidney and cardiac pathology were improved.

It is not presently clear how useful calcium channel blockers will be in the prevention of hypertrophy. As the signalling pathways have not been completely worked out, it is possible that calcium entry from extracellular fluid through L-type calcium channels that are inhibited by calcium channel blockers, may play a role in hypertrophy. In this sense, it is possible that these agents will prevent hypertrophy.

CAPTOPRIL DECREASES MICROALBUMINURIA



Mathiesen et al. BMJ 303:81, 1991.

Figure 35

Dietary Protein Restriction

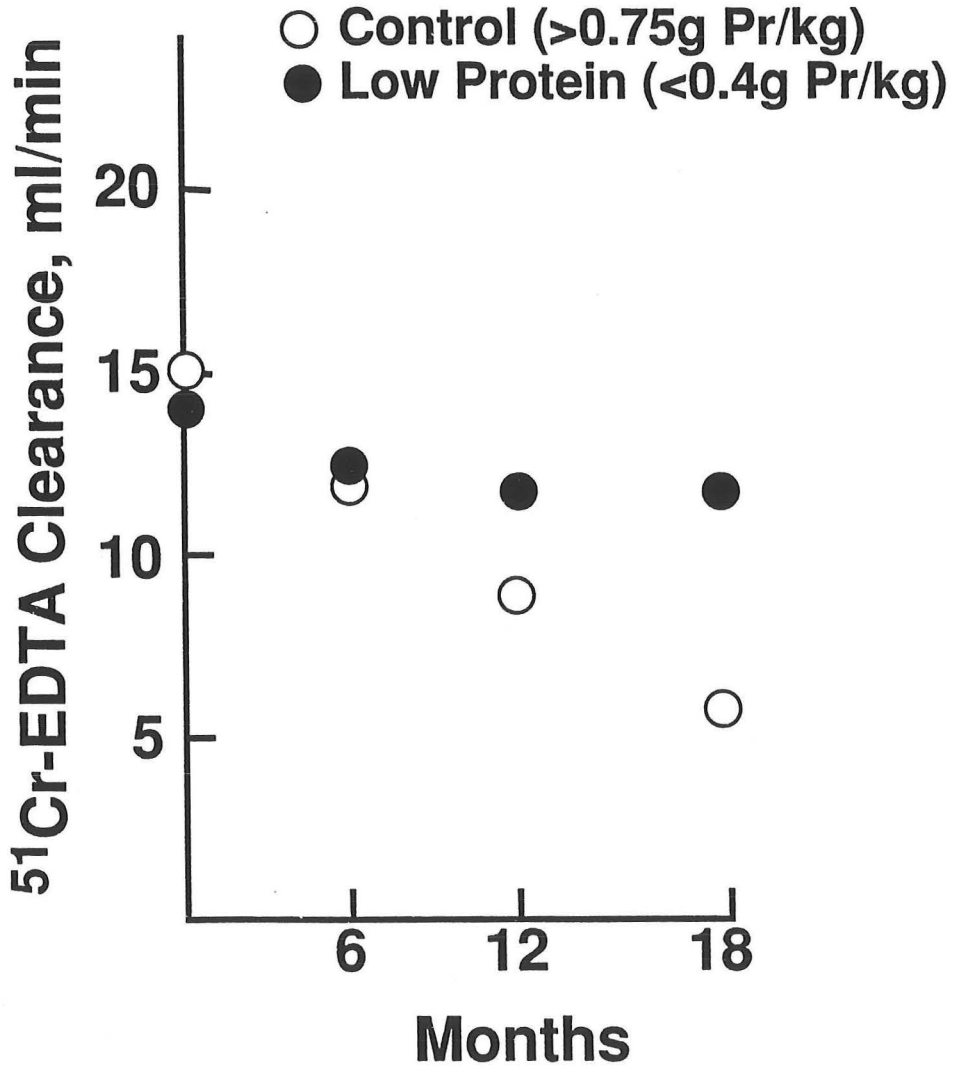
As noted previously, dietary protein restriction reverses hyperfiltration, glomerular hypertension, renal hypertrophy, and renal sclerosis in models of decreased renal mass and diabetic nephropathy. While these results have convincingly demonstrated the usefulness of dietary protein restriction, they have all been performed in rats and the question of whether these results are relevant to humans has been raised. Many studies have been performed examining the effects of dietary protein restriction on the progression of renal disease, but most of these have not been well controlled and prospective. Two recent studies have convincingly demonstrated an effect of dietary protein restriction.

Figure 36 shows the results of Ihle et al (1989) on progression of renal disease in patients with renal insufficiency. Control subjects were placed on a diet containing >0.75 g protein/kg/day, while low protein diet contained <0.4 g protein/kg/day. In the patients on the low protein diet, the proteins ingested were of high biological value. As shown in the figure, while both groups started with similar GFRs of approximately 15 cc/min, the control group lost renal function at a faster rate, while the low protein group stabilized renal function after 6 months.

A similar study was performed by Zeller et al (1991) in diabetic subjects (Figure 37). All patients had diabetic nephropathy with initial glomerular filtration rates between 15 and 85% of predicted values. GFR measured as ^{125}I-labelled iothalamate clearance was then followed. Control patients ingested at least 1 g protein/kg/day, while treated subjects ingested <0.6 g protein/kg/day. As can be seen in the figure, patients on the low protein diet (A) lost renal function at a slower rate than patients on the control diet (B).

Thus, both of these studies demonstrate a beneficial effect of low dietary protein on progression of renal disease. This appears to be true in diabetic nephropathy and in patients with renal insufficiency of varying causes.

DIETARY PROTEIN RESTRICTION SLOWS PROGRESSION OF RENAL DISEASE



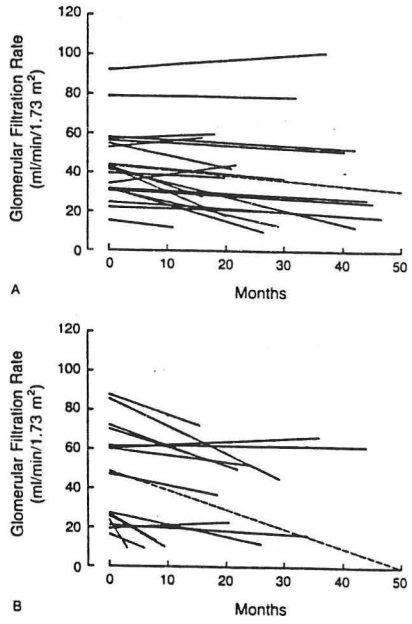
Ihle et. al. NEJM 321:1773, 1989

Figure 36

Dietary Salt Restriction

As noted above, severe dietary NaCl restriction prevents renal hypertrophy and sclerosis in models of decreased renal mass and hypertension (Daniels and Hostetter, 1990; Lax et al, 1992; Benstein et al, 1990). Of note, hydrochlorthiazide was not effective (Benstein et al, 1990). Future studies will be necessary in patients before firm recommendations can be made.

DIETARY PROTEIN RESTRICTION SLOWS PROGRESSION OF RENAL DISEASE IN DIABETIC NEPHROPATHY
(Type I Diabetes Mellitus)



Zeller et al. N Engl J Med 324:78, 1991.

Figure 37

SUMMARY

Based on the above discussion, a number of conclusions can be drawn. First, hypertrophy is a common clinical entity, especially as it involves the kidney, the heart, and vascular smooth muscle cells. Second, there is accumulating evidence suggesting that hypertrophy is detrimental to organ function in the long term. Third, the mechanisms of cellular hypertrophy are just beginning to be understood. It appears that mechanisms of hypertrophy and hyperplasia are similar.

Lastly, converting enzyme inhibitors and low dietary protein have proven efficacy in patients with cardiac and renal disease. While these treatments appear to be beneficial in slowing the progression of renal disease in diabetics, a potentially more important question is whether they can prevent diabetic nephropathy. While not proven, it is likely that a part of the beneficial effects of these treatments are related to inhibition of hypertrophy of the respective organs. Numerous trials are now ongoing examining the effects of converting enzyme inhibitors and dietary protein modification on progression of cardiac and renal disease. In addition, the possibility that other treatments such as calcium channel blockers or dietary salt restriction may be useful is exciting. Lastly, the recent finding of the importance of endothelin as a vasoconstrictor and a growth factor, and the production of receptor blockers and the possible future production of converting enzyme inhibitors, raises the exciting prospect of new classes of agents which may be useful in the treatment of these disorders.

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