Myocardial Remodeling in Hypertensive Ren-2 Transgenic Rats

F.J. Villarreal, D.A. MacKenna, J.H. Omens, W.H. Dillmann

Abstract  Rats harboring the mouse Ren-2 transgene develop hypertension despite low levels of plasma renin. We determined the extent of left ventricular remodeling present in Ren-2 rats at 16 weeks of age by measuring blood pressure, ratio of heart weight to body weight, left ventricular wall thickness, passive (diastolic) left ventricular compliance, and left ventricular collagen content using hydroxyproline and collagen area fraction. Changes in perivascular fibronectin and collagen type I and III were examined with immunohistochemistry. Blood pressure values at time of death were 244±15 mm Hg for Ren-2 rats (mean±SD, n=5). Ratios of heart weight to body weight (grams per kilogram) for Ren-2 animals were 4.1±0.2 versus 3.1±0.1 for controls (n=6, P<.001). Wall thickness values for control animals were 2.6±0.1 versus 4.1±0.4 mm for Ren-2 animals (P<.001). Left ventricular Ren-2 hydroxyproline measurements were significantly decreased (3.4±0.2 versus 4.7±0.9 mg/g dry wt for controls). Significant decreases of approximately 30% were also observed in collagen area fraction in Ren-2 rats. Immunohistochemical and picrosirius red staining indicated increased amounts of perivascular fibrosis in all Ren-2 animals (when compared with controls) with enhanced levels of perivascular fibronectin and type I and type III collagen proteins. Left ventricular compliance measurements indicated a decrease in left ventricular volume for all left ventricular pressures (P<.07). In conclusion, Ren-2 animals at 16 weeks of age demonstrated a substantial degree of cardiac hypertrophy that was accompanied by perivascular but not diffuse fibrosis and a stiffened left ventricle. (Hypertension. 1995;25:98-104.)

Key Words  • angiotensin II • fibrosis • extracellular matrix • transforming growth factor-β • compliance

The development of pathological hypertrophy in the myocardium has been frequently associated with abnormal accumulation of extracellular matrix (ECM) proteins (mostly collagens) in the interstitium.1-3 The abnormal accumulation of fibrillar collagens results in an altered ventricular stiffness4,5 and ultimately may contribute to the development of myocardial failure. Angiotensin II (Ang II) has been implicated as an important modulator of cardiac hypertrophy and interstitial fibrosis.6,7 In vivo studies indicate that the process of cardiac hypertrophy and fibrosis can be modulated through the use of angiotensin-converting enzyme (ACE) inhibitors or nonpeptide Ang II type 1 (AT₁) receptor antagonists such as losartan.8,9 Increased ACE activity has been found in the hypertrophied left ventricle and appears to be associated with altered diastolic properties.10 In vitro studies have also demonstrated the capacity of Ang II to regulate cardiac fibroblast function.11,12 In the clinical setting, the use of ACE inhibitors in patients at risk of developing significant cardiac remodeling leads to the reduction of morbidity and mortality associated with cardiac failure.13

The ECM proteins fibronectin and collagen types I and III are important constituents of the cardiac interstitium that are synthesized by the cardiac fibroblast.14 Collagens I and III constitute the most abundant forms of interstitial myocardial fibrillar collagens.8 The relative proportions of type III and type I can be altered in the hypertrophied rat myocardium,15 and these changes have been correlated to altered myocardial compliance.16 Fibronectin is an ECM and plasma protein that is involved in functions such as cell adhesion and migration17 and has been postulated to play important roles in tissue remodeling.18 The family of regulatory factors known as transforming growth factor-β (TGF-β) has been implicated as a major regulator of ECM production.19 Detectable levels of TGF-β1 and TGF-β3 have been observed in rat myocardium, and enhanced levels appear to be associated with areas of active ventricular remodeling.20,21 We have recently demonstrated the upregulation of TGF-β1 mRNA levels in response to acute ascending aortic banding in rats.22 This increase preceded those of fibronectin and collagen types I and III, suggesting that this regulatory factor might be involved in the process of myocardial remodeling.

Recently, a transgenic rat model was developed that overexpresses the mouse renin (Ren-2) gene.23 The overexpression of this gene leads to the development of severe hypertension. The highest levels of Ren-2 (renin) mRNA and protein were observed in the adrenal gland of the animal with low levels in the kidney.24 Treatment of these animals with ACE inhibitors25 or losartan26 leads to a significant reduction in blood pressure levels. Plasma levels of renin and other components of the renin-angiotensin system are suppressed in these animals, whereas prorenin levels are increased.23 Adrenalectomy in these animals leads to a significant decrease in blood pressure levels,27 supporting the contention that hypertension in Ren-2 rats might be derived at least partly from the overproduction of adrenal renin and stimulation of adrenal steroid release. Indeed, high levels of urinary excretion of adrenal steroids are observed.28 Recent studies performed in the isolated hind-
limb of Ren-2 rats indicate that the vasculature of these animals appears to be capable of overproducing Ang II.\textsuperscript{20} This overproduction of Ang II appears to be coupled to the expression of Ren-2 in the vascular tissue. However, little is known about the expression of the Ren-2 in other tissues, including the heart.

The availability of this transgenic rat offers an opportunity to examine the process of cardiac remodeling in an animal model that not only demonstrates high levels of blood pressure but appears to derive this alteration from the overexpression of renin, a key component of the renin-angiotensin system. To accomplish this goal, we (1) examined the degree of cardiac hypertrophy and left ventricular (LV) chamber compliance, (2) examined changes in myocardial gene expression, and (3) determined the extent of altered deposition of ECM proteins in the Ren-2 rat model.

Methods

Animal Model

A group of five male transgenic Ren-2 (TGmRen-2)\textsuperscript{32} rats were studied at 16 weeks of age. Sprague-Dawley rats (n=6) of similar age and weight were used as controls. Blood pressure measurements were obtained using a tail-cuff apparatus. Body weight, total heart weight, and LV wall thickness measurements were obtained at the time of death.

Pressure-Volume Curves

By use of a method similar to that described by Omens et al.\textsuperscript{30} rats were anesthetized with sodium pentobarbital (100 mg/kg) and ventilated with air. The heart was exposed with a medial sternotomy, the ascending aorta clamped, and the heart arrested with 1 to 3 mL of a hypothermic, hyperkalemic modified Krebs-Henseleit solution by direct injection through the apex into the LV. The arresting solution contained (in grams per liter) NaCl 4.0, KCl 4.8, NaHCO\textsubscript{3} 1.0, glucose 2.0, 2,3-butanediol monoacetate 3.0; and heparin 10 000 U/L, 2,3-Butanediol monoacetate was added to the arresting solution to delay the onset of ischemic contracture. The right and left ventricles were vented, and a fluid-filled balloon attached to a cannula was placed in the LV and secured with a purse-string suture in the mitral annulus. The balloon was connected to a Statham P23ID pressure transducer and volume infuson pump. The ventricular balloon was inflated at a constant rate of approximately 1 mL/min to a peak pressure of 25 to 30 mm Hg, followed by a deflation to the original volume. This "preconditioning" cycle was repeated until the volume at 25 mm Hg was reproducible (within 5% of the previous cycle). Preconditioning typically took two to three cycles, after which the final pressure-volume data were recorded. The LV pressure data were fitted by least-squares regression as a third-order polynomial function of LV volume, and volumes were interpolated at six equal pressure increments from 0 to 25 mm Hg. After data acquisition, sections of the LV were obtained for collagen concentration measurements, picrosirius red staining, immunohistochemistry, and total RNA extraction.

RNA Extraction, cDNA Probes, and Northern Blot Analysis

Total RNA was extracted from rat LV myocardium following a modified procedure of Chomczynski and Sacchi.\textsuperscript{31} A 4-mol/L solution of guanidine isothiocyanate was added to approximately 200 mg of freshly isolated myocardium. A phenol-chloroform extraction was performed, and the aqueous phase was transferred to a new tube after centrifugation at 10 000g at 4°C for 20 minutes. Total RNA was precipitated through two consecutive ethanol precipitations separated by an additional phenol-chloroform extraction step. Quantification of final RNA yield was carried out using UV spectrophotometry at 260 nm. For Northern blot studies, 10 μg of total RNA was electrophoresed on 1% agarose gels and subsequently processed following an established protocol.\textsuperscript{25} RNA was blotted onto nylon membranes and baked in an 80°C vacuum oven for 90 minutes. Filters were hybridized for 24 hours at 42°C using random-priming radiolabeled cDNA probes for fibronectin, α\textsubscript{1} (I) collagen, α\textsubscript{1} (III) collagen, and sarcomplastic reticulum Ca\textsuperscript{2+} ATPase (SERCa\textsubscript{2+}). In the case of TGF-β1 and TGF-β3, hybridization was performed as described by Church and Gilbert\textsuperscript{33} to avoid cross-hybridization to 28S and 18S. Rat cDNA for fibronectin was provided by Dr K. Boheler. This cDNA is directed to the NH\textsubscript{2}-terminal of fibronectin and recognizes all forms of fibronectin. Rat collagen-α\textsubscript{1} (I) containing 900 bp of the 3' noncoding and coding regions was provided by Dr Genovese, and mouse collagen-α\textsubscript{1} (III) probe of 500 bp in length for the coding region was provided by Dr Y. Yamada. Rat cDNA probes for TGF-β1 (985 bp coding region) and mouse TGF-β3 (1200 bp coding region) were provided by Dr M.B. Sporn. A 1-kb rat cDNA was used for SERCa\textsubscript{2+}. Northern blots were subsequently exposed to Kodak XAR radiographic film at −70°C by using intensifying screens. The 28S bands visualized from the etidium bromide photograph were used (scanned) to correct for loading differences. Data are expressed as the ratio of mRNA to 28S signal. Densitometry was used for the purpose of quantification and comparison of relative amount of mRNA autoradiographic signals. Autoradiograms were scanned using digitally captured video images with a Macintosh Centris 650 and a Vicon computer-controlled display camera with a Nikon Micro Nikkor 60-mm lens. Densitometric analysis was performed using National Institutes of Health image software. Different exposures of all autoradiograms were obtained to ensure that scanning was performed within the linear range of densitometry.

Collagen Concentration

Collagen concentration was measured by the hydroxyproline assay,\textsuperscript{35} assuming that collagen contains 14% hydroxyproline. Heart tissue was dried to a constant dry weight by lyophilization and pulverized in a blender. Samples (30 to 40 mg) were hydrolyzed in 6 N HCl in triplicate for 24 hours at 110°C. Collagen concentration was estimated using a colorimetric method as described by Woessner.\textsuperscript{34}

Collagen Area Fraction

To quantify the collagen area fraction of LV fibrillar collagen, an equatorial ring of the LV was fixed in glutaraldehyde and embedded in paraffin. Sections were obtained parallel to the epicardial tangent plane from the LV free wall. At least three sections 10 μm thick were obtained at the midwall. The sections were stained with picrosirius red (Sirius red F3BA; a semiautomated method as described by MacKenna and Omens\textsuperscript{36}) was used to quantify the collagen area fraction based on enhanced birefringence of the collagen seen with picrosirius-polarization microscopy. Briefly, 25 views were selected randomly from the central portion of the section. Under high power (×400) and linear cross-polarized light, each of the 25 views was rotated until the autobirefringence of the muscle was extinguished. Color images of both the brightfield and polarized views were captured using a Data Translation DT-2255 A/D board on a Macintosh Quadra 900. The RGB color images were transformed into hue, saturation, and brightness channels. To find the area of collagen in the polarized view, the brightness channel was used to provide a threshold limit such that only the birefringent collagen was visible. This mask was then applied to the hue channel, leaving a spectrum of 256 colors representing the number of pixels of birefringent collagen at 256 hue frequencies. The area of tissue was found by thresholding the blue channel of the brightfield image, and the area fraction of collagen was found by normalizing the number of pixels at each color level by the total tissue pixels. Finally, the
functional range (red to teal) of the 256 color levels were subgrouped into five groups, each comprising approximately 12% of the total spectrum. These subgroups represented collagen with a median color of red, orange, yellow, green, and teal. Because the size of the collagen fibers plays a major role in the polarization colors seen with this method, some extent these color groupings correspond to the relative sizes of the collagen fibrils, with red being the largest and teal being the smallest. In all cases, regions with blood vessels or significant preparation artifact were avoided.

**Immunohistochemistry**

Ring sections of LV myocardium were immersion-fixed in 4% buffered formaldehyde for a period of 4 hours. After fixation, LV myocardium was embedded in paraffin, and 10-μm sections were made. A streptavidin/alkaline phosphatase-based protocol with Vector Red (Vector Labs) was used for immunohistochemically staining our LV sections. This method enhances the effects of immunostaining by yielding a red reaction that is also visible under fluorescent microscopy with rhodamine and fluorescein lights. Blocking was performed with 10% serum (from the animal source of the secondary antibody to be used) in 0.1% bovine serum albumin/Tris-buffered saline. Primary antibodies were subsequently added and incubated for 60 minutes at room temperature. Preimmune sera of the animal species in which the primary antibody was developed were used as negative controls. Biotinylated immunoglobulin G was used as the secondary antibody, and incubation was continued for 60 minutes. This step was followed by incubation with alkaline phosphatase-conjugated streptavidin for 30 minutes. To quench any endogenous fibroblast phosphatase activity, levamisole was included as part of the mixture of developing reagents.

**Data Analysis**

Unpaired t-tests were used for statistical comparisons of physiological parameters, gene expression, and collagen concentration. For comparison of pressure-volume data and collagen area fraction, a repeated-measures ANOVA was used.

**Results**

The average body weight was similar for the two groups: 470±30 g for Ren-2 versus 460±28 g for controls. Mean heart weight was significantly greater in Ren-2 animals (1.9±0.1 g) than in the control animals (1.4±0.06 g; P<.001). Consequently, ratios of heart weight to body weight (grams per kilogram) for Ren-2 animals were significantly greater than for controls.

![Graph](image)

**Fig. 1.** Graph shows left ventricular pressure-volume relation computed from arrested hearts of age- and weight-matched controls (n=6) and 16-week-old Ren-2 rats (n=5). Data are expressed as mean±SD.

Fig 2. Northern blot analysis of total cardiac RNA obtained from control and from Ren-2 hypertrophied rat hearts. Ten micrograms of total RNA was loaded per lane. For this example the Northern blot was initially probed with cDNA for transforming growth factor (TGF)-β1, subsequently for TGF-β3, and finally with SERCa2+. As a reference, 18S and 28S rRNA correspond to approximately 1.7 and 4.85 kb, respectively. TGF-β1 corresponds to 2.5 kb, TGF-β3 to 3.0 kb, and SERCa2+ to 4.5 kb.

(4.1±0.2 versus 3.1±0.1; P<.001). Wall thickness values for control animals were 2.6±0.1 versus 4.1±0.4 mm for Ren-2 animals (P<.001). Mean blood pressure at time of death was 244±15 mm Hg for Ren-2 rats.

A repeatable pressure-volume relationship was found in all hearts after two to three preconditioning runs. The mean pressure-volume curves during the inflation portion of the cycle are given in Fig 1. The volumes given include the measured residual volume taken up by the empty balloon and cannula in the unloaded LV. A two-way ANOVA indicated that, although there was a decrease in Ren-2 LV volume at all pressures (leftward shift in the pressure-volume relation), it was not significantly different from that in control animals (P=.07). The interaction between the pressure variable and treatment was also not significant (P=.38), suggesting that there was a uniform decrease in volumes at all pressures (ie, no change in Ren-2 animal pressure-volume curve slope).

Fig 2 illustrates the changes observed in gene expression for TGF-β1, TGF-β3, and SERCa2+. As can be seen in the figure of the autoradiograms, Ren-2 animals demonstrated increases in mRNA levels for TGF-β1, no changes in TGF-β3, and a decrease in the levels of SERCa2+. As shown in the Table, statistical analysis showed significant increases in TGF-β1 and collagen type I, with significant decreases in fibronectin and SERCa2+ mRNA levels. There was a tendency for lower levels of type III collagen gene expression in Ren-2 animals, but this difference was not significant.

**Summary of Changes in Myocardial Gene Expression in Control and Ren-2 Rats**

<table>
<thead>
<tr>
<th>mRNA Species</th>
<th>mRNA/28S Control</th>
<th>mRNA/28S Ren-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β1</td>
<td>0.86±0.08</td>
<td>1.12±0.19*</td>
</tr>
<tr>
<td>TGF-β3</td>
<td>0.61±0.39</td>
<td>0.63±0.09</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>0.81±0.23</td>
<td>0.39±0.22*</td>
</tr>
<tr>
<td>Collagen I</td>
<td>0.94±0.33</td>
<td>1.5±0.40*</td>
</tr>
<tr>
<td>Collagen III</td>
<td>1.6±0.27</td>
<td>1.2±0.46</td>
</tr>
<tr>
<td>SERCa2+</td>
<td>3.5±0.42</td>
<td>1.0±0.95†</td>
</tr>
</tbody>
</table>

TGF indicates transforming growth factor.

*Statistical significance at P<.05.
†Statistical significance at P=.001.
Collagen concentration as determined by a hydroxyproline assay was significantly decreased ($P<.01$) in Ren-2 animals ($3.4\pm0.1$ mg/g dry wt) versus controls ($4.7\pm0.9$ mg/g dry wt). Similarly, interstitial collagen area fraction was decreased ($P<.01$) in Ren-2 rats compared with controls (Fig 3), particularly in the larger orange fibers ($P<.01$). The picrosirius red staining revealed substantial perivascular fibrosis in all of the hypertensive Ren-2 animals. A representative example of these observations is shown in Fig 4. Whereas images obtained from control animals show a limited distribution of perivascular collagen, images from Ren-2 animals illustrate a pattern of centrifugal deposition of collagen. These observations were further corroborated by the use of immunohistochemical staining as shown in Fig 5. Sections obtained from control animals showed little immunoreactivity around the blood vessel with antibodies directed against fibronectin, collagen I, or collagen III. On the other hand, Ren-2 animals had an enhanced perivascular deposition of these ECM proteins, in some cases extending beyond the vascular area.

**Discussion**

Results from this study indicate that Ren-2 rats at 4 months of age demonstrate a significant degree of cardiac hypertrophy. This phenomenon was accompanied by a stiffening of the LV as shown by the change in the pressure-volume curve. This reduced LV compliance does not appear to be associated with an enhanced deposition of interstitial ECM proteins, given that fibrillar collagen is reduced at this particular stage of disease. There was, however, substantial wall thickening and perivascular fibrosis, which may contribute to an increased LV stiffness despite the reduced interstitial fibrosis. This phenomenon was accompanied by enhanced gene expression for TGF-β1 and for that of type I collagen.

Ren-2 transgenic rats develop fulminant hypertension as a consequence of the overexpression of the mouse renin gene. It has been shown that untreated animals develop hypertension-related alterations and pathological lesions in a variety of tissues, including the kidney (sclerosis) and vasculature (hypertrophy of the media and remodeling). Preliminary information also indicated that these animals develop cardiac hypertrophy and perivascular fibrosis. Characterizing cardiac hypertrophy in these animals would eventually allow us to

Fig 3. Bar graph shows interstitial collagen area fraction measured with the picrosirius-polarization technique in control and 16-week-old Ren-2 rats. Collagen fiber size decreases from red (large) to teal (small). Total collagen area fraction is represented as the sum of all colors (right side of graph). Significant differences from control ($P<.01$) are denoted by an asterisk (*).

Fig 4. Photomicrographs of picrosirius red–stained rat left ventricular tissue sections show left ventricular interstitial fibrillar collagen for a control (A) and a Ren-2 rat (B) and perivascular collagen deposits in a control (C) and a transgenic (D) rat.
determine the suitability of this animal model for exploring the in vivo roles of pressure overload, enhanced Ang II, or aldosterone formation in myocardial remodeling. We documented the changes that accompany these alterations in the myocardium in 4-month-old animals and compared them to normal (normotensive) control rats. Results indicated that Ren-2 rats at this stage demonstrate a significant degree of cardiac hypertrophy. The extent of cardiac hypertrophy corresponds to an increase in mass of about 30%. This observation was accompanied by an increased wall thickness and reduced unloaded LV volume. Spontaneously hypertensive rats of comparable age (13 weeks) demonstrated blood pressure levels of approximately 200 mm Hg with average ratios of heart weight to body weight of about 3.9. Analysis of changes in myocardial TGF-β gene expression in these animals indicated increased levels of mRNA for TGF-β1 with unchanged levels for TGF-β3. These results are similar to those observed in our rat model of aortic banding–induced cardiac hypertrophy. Increased levels of TGF-β1 mRNA have also been observed in response to cardiac hypertrophy induced with abdominal aortic banding in rats. In this animal model (abdominal banding), treatment with losartan abolished cardiac hypertrophy and normalized TGF-β1 mRNA levels. However, by the evidence presented in
the abdominal-banded model discussed above or from our Ren-2 rats, it is unclear if enhanced levels for TGF-β1 in the myocardium are a reflection of cardiac injury, pressure overload, or enhanced levels of tissue Ang II. In our Ren-2 animals, levels of TGF-β3 gene expression were unchanged, suggesting that the role of this growth factor in the process of myocardial remodeling remains unclear. We also examined changes in fibronectin gene expression and found that fibronectin mRNAs were lower than those of control animals. This is surprising in light of the fact that fibronectin is significantly induced by TGF-β1. Other studies have indeed suggested an association between the overexpression of TGF-β1 and fibronectin deposition in the myocardium. Collagen type I mRNA levels were significantly upregulated in this animal model, whereas those of type III decreased but not significantly. The regulation of type III collagen mRNA levels in Ren-2 animals appears to differ with respect to other animal models. Interestingly, preliminary observations (data not shown) using high-performance liquid chromatography analysis of collagen types indicate higher levels of type III collagen in Ren-2 rats versus control animals. We also examined the changes in gene expression for the SERCA2+. The product of this gene is an important regulator of Ca2+ reuptake and thus relaxation in the cardiac myocyte. It is thought that ventricular dysfunction, such as that seen in failure, might be due at least in part to the downregulation of SERCA2+ levels. In our previous study, we observed a downregulation of this gene of about 30% in aortic-banded rats.

We also examined collagen deposition in LV tissue of these animals. Surprisingly, results using both hydroxyproline and collagen area fraction indicated reduced levels of interstitial collagen for these animals. These results are in disagreement with the mRNA analysis. It is therefore possible that at this stage of their pathology there is an enhanced degree of collagen pre- and post-translational processing (turnover). In a recent study performed by Eleftheriades et al., the in vivo rates of myocardial procollagen synthesis, collagen accumulation, and intracellular procollagen degradation were assessed in abdominally banded rats. Their measurements also were correlated with myocardial mRNA levels for collagen types I and III. In banded rats with cardiac hypertrophy, increases in type III collagen mRNA were noted immediately after banding, and levels remained high thereafter, whereas type I collagen mRNA increased only after 16 weeks of banding. The development of interstitial fibrosis, however, was observed after 16 weeks of banding. This illustrates the importance of considering not only the rates of procollagen synthesis but also the rapid degradation of newly synthesized procollagens to determine the final amount of collagen accumulation. It is therefore possible that in the present study Ren-2 animals had greater degradation of procollagens, leading to a net decrease in collagen accumulation. There was a clear increase in perivascular ECM in Ren-2 animals, as shown by both picrosirius red-stained sections and by immunohistochemistry directed against fibronectin and collagen types I and III. This perivascular fibrosis has also been observed in a variety of animal models in which cardiac hypertrophy develops as a result of hypertension associ-ated with the activation of the renin-angiotensin system.

LV volume was decreased for Ren-2 animals compared with the controls. Other models of hypertrophy with comparable decreases in LV volume often reveal increases in myocardial collagen. The Ren-2 rats had decreased collagen density and potentially a decrease in total LV collagen after correction for tissue mass. Thus, the increased stiffness of the LV chamber cannot be attributed to this phenomenon. Recent mathematical modeling performed in our laboratory indicates that changes in LV geometry following pressure overload may account for much of the decrease in LV volume independent of changes in myocardial material properties. Additionally, changes in properties of the myocyte (sarcomere or cytoskeletal structures) may also contribute to the increased stiffness. We cannot, however, exclude the presence of tissue edema as a potential contributor of altered stiffness due to the unavailability of wet/dry heart weights.

Thus, this animal model of cardiac hypertrophy has shown unique properties with respect to the ECM protein components. The data presented in this study represent only one time point in the natural history of myocardial remodeling in Ren-2 rats. Although there is evidence for enhanced production of Ang II in the vascular tissue of these animals, a careful characterization of myocardial Ang II levels has not been performed. Extending the characterization of ventricular remodeling beyond that done in this study and determining the local tissue levels of Ang II might be necessary to determine whether the process of cardiac enlargement in Ren-2 rats derives solely from pressure overload or from its combination with the enhanced production of Ang II.

Acknowledgments

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References


