British Journal of Pharmacology (2001) 134, 977–984

www.nature.com/bjp

Renoprotective effects of carvedilol in hypertensive-stroke prone rats may involve inhibition of TGFβ expression

[1]Victoria Y. Wong, [1]Nicholas J. Laping, [2]Allen H. Nelson, [1]Lisa C. Contino, [1]Barbara A. Olson, [1]Eugene Gygielko, [3]Wallace G. Campbell Jr., [2]Frank Barone & *,[1]David P. Brooks

[1]Department of Renal Pharmacology, GlaxoSmithKline, King of Prussia, Pennsylvania, PA 19406, U.S.A.; [2]Department of Cardiovascular Pharmacology, GlaxoSmithKline, King of Prussia, Pennsylvania, PA 19406, U.S.A. and [3]Department of Pathology, Emory University School of Medicine, Atlanta, Georgia, GA 30322, U.S.A.

1 The effect of carvedilol on renal function, structure and expression of TGFβ and the matrix proteins fibronectin, collagen I and collagen III, was evaluated in spontaneously hypertensive stroke-prone (SHR-SP) rats fed a high fat, high salt diet.

2 Carvedilol treatment for 11 to 18 weeks did not alter systolic blood pressure in SHR-SP rats, however, it resulted in a significant reduction in heart rate.

3 Carvedilol treatment reduced renal fibrosis and total, active and chronic renal damage to levels approaching those of WKY rats on a normal diet.

4 Urinary protein excretion was higher in SHR-SP rats (51 ± 10 mg day^{-1}) than WKY rats (18 ± 2 mg day^{-1}) and this was further increased when SHR-SP rats were fed a high fat, high salt diet (251 ± 120 mg day^{-1}). Treatment with carvedilol resulted in significantly lower urinary protein excretion (37 ± 15 mg day^{-1}).

5 The expression of TGFβ mRNA was significantly higher in SHR-SP rats compared to WKY rats and a further increase was observed when rats were fed a high fat, high salt diet. Renal TGFβ expression was significantly reduced by treatment with carvedilol. The expression of fibronectin and collagen I and collagen III mRNA showed a pattern similar to that observed with TGFβ mRNA expression. Collagen I mRNA expression followed a pattern similar to renal fibrosis.

6 These data indicate that carvedilol can provide significant renal protection in the absence of any antihypertensive activity and that the mechanisms involved in this action may include reduced expression of profibrotic factors such as TGFβ.

British Journal of Pharmacology (2001) **134,** 977–984

Keywords: Transforming growth factor; renal disease; beta blocker

Abbreviations: SHR-SP, spontaneously hypertensive-stroke prone; TGFβ, transforming growth factor β; WKY, Wistar Kyoto

Introduction

Carvedilol is a vasodilating beta blocker with antioxidant activity that is being used very successfully in patients with congestive heart failure (Packer *et al*., 1996; Cleland, 1998). Carvedilol has also been shown to provide significant renal protection in the partial nephrectomy rat model of progressive renal disease (Brooks *et al*., 1993; Rodriguez-Perez *et al*., 1995; Nakamoto *et al*., 1988); indeed in one study it demonstrated equal efficacy to captopril (Brooks *et al*., 1993), which is one of the few drugs shown to be effective in patients with renal disease (Lewis *et al*., 1993). The renoprotective effects of carvedilol in the 5/6 nephrectomized rats were, for the most part, accompanied by reductions in blood pressure suggesting that carvedilol's antihypertensive activity was an important mechanism in the beneficial effects observed. Studies in spontaneously hypertensive stroke-prone (SHR-SP) rats, however, have indicated that carvedilol can reduce renal damage without lowering blood pressure, suggesting that other renoprotective mechanisms may be involved (Barone *et al*., 1996). Since there is growing evidence that renal fibrosis is important in the progression of renal disease, we have evaluated the effects of carvedilol, at a dose that did not lower systolic blood pressure, on the mRNA levels of the profibrotic cytokine, transforming growth factor beta (TGFβ), the matrix proteins fibronectin and collagens I and III, and renal histopathology including fibrosis in SHR-SP rats.

*Author for correspondence at: GlaxoSmithKline, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939, U.S.A.
E-mail: David_P_Brooks@gsk.com

Methods

Experimental design

SHR-SP, progeny from the strain developed by Okamoto *et al*. (1974), were obtained from the National Institutes of Health (Bethesda, MD, U.S.A.) and were bred in the Department of Laboratory Animal Science at SmithKline Beecham Pharmaceuticals (King of Prussia, PA, U.S.A.). Animals were housed and cared for in accordance with the Guide for the Care and Use of Laboratory Animals [DHEW (DHHS) Publication No. (NIH) 85-23, revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205]. Procedures using laboratory animals

were approved by the Institutional Animal Care and Use Committee of SmithKline Beecham Pharmaceuticals. Male SHR-SP between 10 and 12 weeks of age were adapted to individual cages and fed powdered NIH-07 diet for 2 weeks.

SHR-SP rats were assigned to one of three groups on the basis of body weight and age (i.e., both parameters were balanced equally between groups) as described previously (Ogiku *et al*., 1993). Two groups received 1% NaCl as drinking water *ad libitum* and the NIH-07 diet (Na + % = 0.33; K + = 0.80%) supplemented with 24.5% fat (high-fat) diet as described previously (Ogiku *et al*., 1993), but one was supplemented with 2400 p.p.m. carvedilol (SK&F 105517; 1-(9H-carbazol-4-yloxy)-3[[2-methoxyphenoxy)ethyl]amino]-2propranol) synthesized at SmithKline Beecham Pharmaceuticals. The remaining SHR-SP group and the normal WKY rats received the normal diet and tap water. All diets were milled and formulated by Zeigler Brothers, Inc. (Gardners, PA, U.S.A.). Rats were treated for 18 weeks. When SHR-SP rats on high fat, high salt diet became moribund prior to the end of the 11th week, they and their match-paired carvedilol-treated SHR-SP rats on high fat, high salt diet were sacrificed. Tissues were studied histologically. The complete experimental was performed on rats surviving for 12 to 18 weeks or more, using a set-sacrifice-protocol, i.e., simultaneously sacrificing one animal from each of the four groups at regular intervals.

Determination of physical and biological parameters

Blood pressure and heart rate were monitored at 6, 8 and 16 weeks using a tail cuff with a IITC Life Science model 179 BP Apollo Analyzer (Woodland Hills, CA, USA), however, since there were no differences over time, only the 16-week data are shown. Urinary protein excretion was determined by placing rats in metabolism cages in order to collect 24 h urine samples, which were immediately stored at −20°C until assay. Urinary protein concentration was determined using the sulfosalicylic acid method (Davidsohn & Henry, 1969) and 24 h urinary protein excretion calculated. At the conclusion of the study, blood samples were taken and serum urea nitrogen and serum creatinine concentration determined using a clinical analyser.

Kidney histopathology

Tissue samples of 5–8 rats from each of the experimental groups were prepared for morphological examination. Whole body perfusion with 200 ml phosphate buffered saline (pH 7.2) was conducted immediately upon euthanasia using an overdose of pentobarbital. Kidneys were removed and appropriate samples stored in formalin. A standard central coronal transverse section of each kidney was then processed histopathological evaluations. After dehydration and processing into paraffin, sections were cut at 6 microns and stained using Hematoxylin and Eosin (H&E) and Masson trichome (MT) techniques as described in detail previously (Luna, 1986). Multiparametered histopathological evaluations were then performed on standard sections. Standard central transverse H&E sections were used to determine kidney crude scores and other parameters of tissue damage. Adjacent MT sections were used to evaluate fibrosis.

Crude scores of renal damage were determined as described in detail previously (Volpe *et al*., 1990; Camargo *et al*., 1993; Barone *et al*., 1996) on standard H&E sections. Briefly, standard transverse sections were graded based on overall renal damage as to the presence of arterial proliferative changes, arterial necrosis, glomerular lesions, mostly proliferative, but also sometimes thrombotic, necrotic and sclerotic, and foci of tubular regeneration, interstitial nephritis, tubular casts and interstitial fibrosis, as follows: 0 (no damage), 0.5 (rare early arterial/arteriolar proliferation and/or necrosis and/or mild tubulo-interstitial changes), 1 (necrosis and/or proliferation of a few arterioles with focal regeneration and mild interstitial inflammation), 2 (moderate arterial/arteriolar necrosis and proliferation with secondary glomerular changes tubular regeneration and interstitial inflammation and or fibrosis in up to half of the cortical and medullary parenchyma) or 3 (extensive necrosis and proliferation of arteries/arterioles with secondary glomerular changes, including infarcts, and regenerative tubular changes and interstitial inflammation and fibrosis in over half of the parenchyma). Ties were broken, as much as possible, by ranking slides at similar levels and making appropriate minor fractional adjustments in scores.

Total active renal damage was determined counting and summing the incidence of each of its four components, the number of arterial lesions, glomerular lesions, foci of tubular changes components, and interstitial inflammation, on the standard H&E section of each rat.

Chronic renal damage or degree of nephron loss was taken to be a reflection of the number of tubular casts in both the cortex and medulla of each standard section evaluated. Chronic arterial, glomerular and tubulointerstitial changes including fibrosis were relatively infrequent in this sample of rats, and consequently were not included in the evaluation of chronic damage.

Foci of normal and pathologic fibrosis were counted on the standard MT-stained kidney section from each rat. Areas of normal fibrosis were mostly confined to periarterial adventitia. Pathologic fibrosis was mainly in the interstitium. Areas of glomerular or arterial fibrosis (scarring) were encountered less frequently.

All histological determinations were made in a completely blind manner (i.e., sections were coded and the analysis of each section was completed without any knowledge of treatment group classification).

RNA extraction and Northern blot hybridization

Saline perfused tissues were immediately dissected, weighed, frozen in liquid nitrogen and stored at −80°C until RNA extraction. Total RNA was prepared from frozen tissues by guanidinium thiocyanate denaturation (Chomczynski & Sacchi, 1987). 10 μg RNA was electrophoresed on 1% agarose gel after glyoxalation for 1 h at 55°C. RNA was transferred to BrightStar-plus nylon membrane (Ambion). Radiolabelled probes were synthesized using the random-primed DECAprime II (Ambion). Hybridization was performed with the radiolabelled probe at 42°C overnight in 50% formamide, 5 × SSPE, 2.5 × Denhardt, 0.1% SDS, 100 μg ml^{-1} salmon sperm DNA and 10 g Dextrans. Membranes were washed with 0.1 × SSPE/0.1% SDS at

65°C and were exposed to a phosphor imaging plate. Data were quantified with ImageQuant software (Molecular Dynamics, Sunnyvale, CA, U.S.A.).

The probe for ribosomal protein L32 (rpL32) was generated by PCR as described in Wang *et al*., 1995. The probe for fibronectin was generated from a 500 bp PCR fragment of fibronectin cDNA and was cloned into the PCR II vector using the TA cloning kit (Invitrogene, CA). The 728 bp PCR fragment of TGF-β1 cDNA was generated from the TGF-β1 full-length clone provided by Dr Nancy Nichols.

Quantitative reverse transcription-PCR

The expression of collagen I and collagen III was evaluated by quantitative Reverse Transcription–PCR using Taqman Realtime PCR 7700 system (PE Applied Biosystems, Foster City, CA, U.S.A.). cDNA was synthesized from 2 μg of total RNA using random hexamer primers and Superscript II reverse transcriptase (Life Technologies, Gaithersburg MD, U.S.A.) and diluted 20 fold. A 25 (L reaction volume containing 200 nM primers, 200 nM probe and Master Mix (PE Applied Biosystems) were mixed with 2 μl diluted cDNA and amplified by PCR. The thermal cycle conditions consisted of initial incubation steps of 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 1 min. The primer sequences for rat collagen I were (probe)-FAM-TTGCATAGCTCGCCATCGCACA-TAMRA, (forward)-TATGCTTGATCTGTATGTGCCACAAT, (reverse)-TCGCCCTCCCGTTTTTG. The primer sequences for rat collagen III were (probe)-FAM-CTTTCCAGCCGGGCCTC-CCAG-TAMRA, (forward)-CAGCTGGCCTTCCTCAGACT, (reverse)-TGCTGTTTTTGCACTGGTATGTAA. The primer sequences for rat RPL 32 were (probe)-TET-AGGCATC-GACAACAGGGTGCGG-TAMRA, (forward)-GAAACTG-GCGGAAACCGA, (reverse)-GGATCTGGCCCTTGAAT-CTTC.

Data analysis

Relative expression levels were determined by comparing cycle times at threshold of samples to those of the standard curve, which was generated with serial dilutions of control kidney cDNA. The 20 fold dilution was arbitrarily set as one. All data are corrected to the relative ribosomal protein L32 (rpL32) mRNA levels. Data are expressed as mean $\pm$ s.e.mean, throughout. Statistical analyses for biochemical data were conducted using an analysis of variance (for repeated measures when appropriate) and subsequently a Scheffe F test.

For histological studies all multiple group comparisons were made by parametric and non-parametric one-way analyses of variance for unpaired data followed by *post hoc* comparisons using Dunn's and Tukey-Kramer Multiple Comparisons Tests and unpaired *t*-tests or the Mann-Whitney U-test corrected for multiple comparisons. Correlations between endpoints were conducted using Pearson's correlation analysis and the Spearman's rank correlation test. All statistical analyses were done using InStat II (GraphPad Software, San Diego, CA, U.S.A.). A probability level of $P<0.05$ (for both parametric and nonparametric analyses of the histological studies) was considered to be statistically significant.

Results

Physical and biological parameters

SHR-SP rats had a significantly higher systolic blood pressure than the normotensive WKY rats (Figure 1). Feeding of a high-fat, high-sodium diet did not significantly alter systolic blood pressure and treatment of these rats with carvedilol also had little effect on blood pressure. However, SHR-SP rats treated with carvedilol had a significantly lower heart rate (Figure 1). The body weight of SHR-SP rats fed a high fat, high sodium diet was slightly but significantly lower than either the SHR-SP or WKY rats on a normal diet. Interestingly, treatment with carvedilol normalized this by mechanisms not well understood. Urinary protein excretion was significantly increased in SHR-SP rats and this was even higher in rats on the high fat, high salt diet. Treatment with carvedilol prevented the enhanced proteinuria (Figure 2). Serum urinary nitrogen was reduced in the SHR-SP rats and this effect was attenuated by carvedilol. Serum creatinine concentration was not significantly altered in SHR-SP rats fed a high fat, high sodium diet, however, carvedilol significantly lowered the serum creatinine concentration (Figure 3). Calculation of creatinine clearance did not demonstrate any statistically significant differences, however, the trend was similar to the changes in serum creatinine (data not shown).

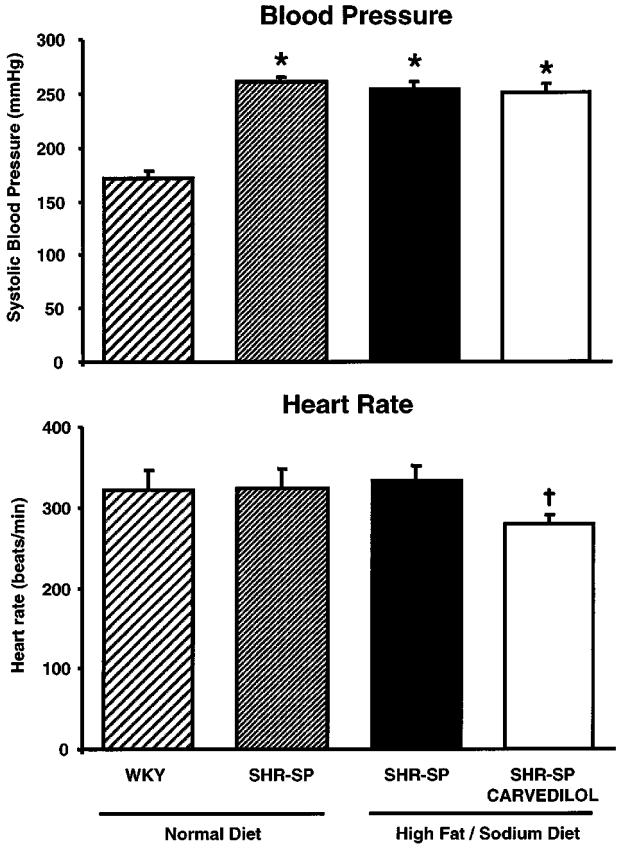

Figure 1 Effect of carvedilol on systolic blood pressure (upper panel) and heart rate (lower panel), in WKY and SHR-SP rats fed a normal or high fat/sodium diet. $n=5-6$ rats/group. $P<0.05$ versus WKY, †$P<0.05$ versus SHR-SP.

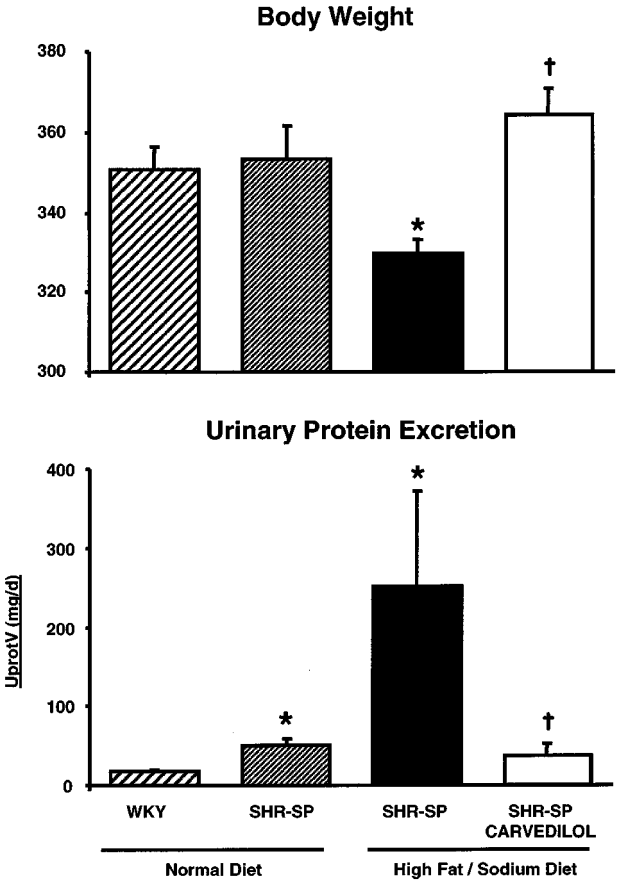

Figure 2 Effect of carvedilol on body weight (upper panel) and urinary protein excretion (lower panel), in WKY and SHR-SP rats fed a normal or high fat/sodium diet. $n=5-6$ rats/group. $P<0.05$ versus WKY, †$P<0.05$ versus SHR-SP.

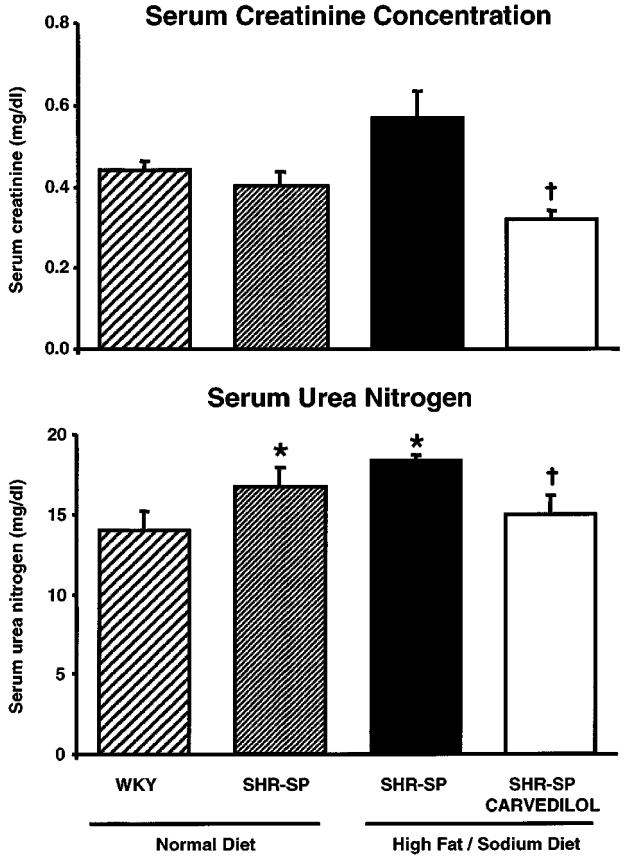

Figure 3 Effect of carvedilol on serum creatinine concentration (upper panel) and serum urinary nitrogen (lower panel), in WKY and SHR-SP ras fed a normal or high fat/sodium diet. $n=5-6$ rats/group. $P<0.05$ versus WKY, $P<0.05$ versus SHR-SP.

Kidney histopathology

Thirty-nine per cent of SHR-SP rats on high fat, high salt diet succumbed during the first 11 weeks of the study. They had advanced renal damage (kidney crude scores, 2.57 ± 0.07) and their matched-pair-sacrificed, carvedilol-treated SHR-SP rats on high fat, high salt diet had none (kidney crude score, 0.01 ± 0.01), $P<0.0001$.

SHR-SP rats sacrificed after 12 or more weeks demonstrated more modest kidney crude scores (Table 1). Carvedilol treatment of SP-SFD rats on high fat, high salt diet reduced renal injury in terms of kidney crude scores, total active renal damage, chronic nephron loss to levels of SP and WKY rats on normal diets (Table 1). The components of total active renal damage for the most part followed similar patterns although those for the components foci of tubular regeneration and interstitial inflammation were less dramatic than those for the components of arterial necrosis (Figure 4), arterial proliferation, and glomerular lesions (Figure 5). Kidney crude scores, total active renal damage and chronic nephron loss were strongly correlated ($P<0.0001$).

Renal fibrosis in SP-SFD rats was elevated about 60 per cent. Carvedilol treatment of SP-SFD reduced renal fibrosis to levels found in WKY normal diet rats (Table 1). Individual kidney sections demonstated 15 ± 1 areas of normal (structural) fibrosis. For all groups, renal collagen I mRNA expression tracked closely with renal fibrosis (see below).

Expression of TGFβ mRNA

The expression of TGFβ mRNA was significantly higher in SHR-SP rats compared to WKY rats (Figure 6). A further increase in TGFβ mRNA levels was observed when the SHR-SP rats were fed a high-sodium diet. This increase in TGFβ mRNA was significantly attenuated by treatment with carvedilol (Figure 6). The expression of fibronectin mRNA, as determined by Northern blot analysis (Figure 7), and collagen I and collagen III mRNA, as determined by quantitative RT-PCR (Figure 8), showed a very similar pattern as observed with TGFβ mRNA expression. Thus, the highest expression of these matrix proteins was observed in SHR-SP rats fed a high fat, high sodium diet and this was significantly attenuated by carvedilol treatment.

Expression of TGFβ mRNA and renal injury

Kidney crude scores, chronic nephron loss, total active renal damage and three of its components, arterial necrosis,

Table 1 Effect of carvedilol (CVD) on histopathology of SHR-SP fed a high fat/high sodium diet

	Normal Diet		*High Fat/Sodium Diet*	
	WKY	*SHR-SP*	*SHR-SP*	*SHR-SP+CVD*
Kidney crude score	0.15±0.04†	0.85±0.17†,*	1.74±0.07#	0.71±0.22†
Total active renal damage	2.0±1.0†	31±8†,*	94±10#	19±10†
Arterial necrosis	0±0†	1.6±0.6†,*	13±3#	1.0±0.7†
Arterial proliferation	0.4±0.4†	15±6†,*	38±4#	10±5†
Glomerular lesions	0.5±0.4†	6±2†,*	31±5#	4.0±2.0†
Foci of tubular regeneration	0.5±0.4#,†	5.8±1.8*	6.4±1.0	1.9±1.3†
Foci of interstitial nephritis	0.9±0.4†	3.1±0.7*	6.6±2.3#	2.4±0.8
Total tubular casts	0.6±0.3†	72±18†,*	350±80#	21±11†
Foci of renal fibrosis	16±1†	20±2	25±1#	15±2†

$n=5-8$ kidneys/group. Units as described in Methods section. #$P<0.02$ versus all other groups; *$P<0.05$ versus WKY; †$P<0.05$ versus SHR-SP on high fat/sodium diet.

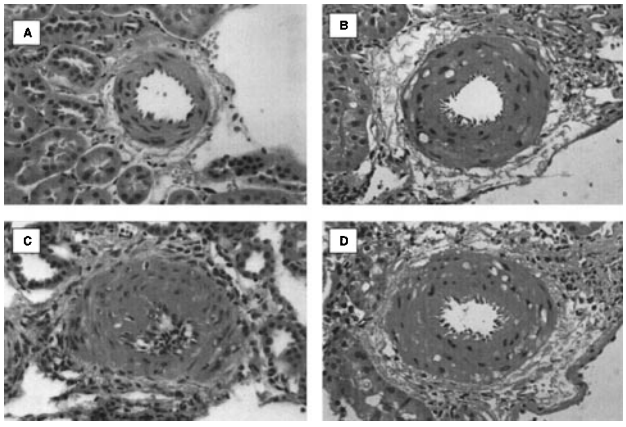

Figure 4 Examples of H&E stained arcuate arteries from a WKY rat; normal (A), an SHR-SP; medial and endothelial cell proliferative changes (B), an SHR-SP fed a high fat/sodium diet; medial and endothelial cell proliferation with subintimal necrosis (C) and an SHR-SP fed a high fat/sodium diet and treated with carvedilol; medial and endothelial cell proliferation (D).

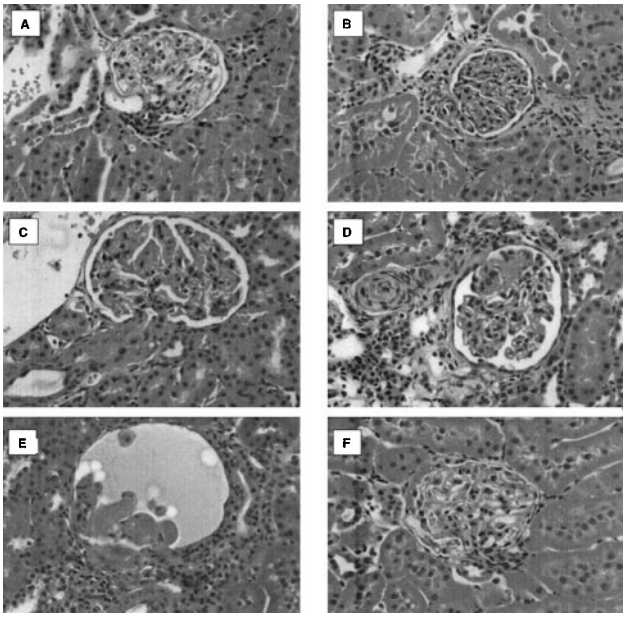

Figure 5 Examples of H&E stained glomeruli from a WKY rat; normal glomerular tuft and afferent and efferent arterioles (A), an SHR-SP; ischemic collapse of glomerular tuft and thickened afferent arteriole (B), three SHR-SP fed a high fat/sodium diet (C, D & E); hypercellular glomerular tuft with necrosis of afferent arteriole. Focus of interstitial fibrosis, right. (C); focal necrosis of glomerular tuft, above, and proliferative changes of adjacent afferent arteriole, left. Focus of interstitial inflammation, lower left. (D); fibrin thrombi in glomerular capillaries and dilatation of Bowman's space (E) and an SHR-SP fed a high fat/sodium diet and treated with carvedilol; normal glomerular tuft and afferent and efferent arterioles (F).

proliferative arterial lesions, glomerular lesions were strongly correlated with renal expression of TGFβ mRNA ($P<0.0035$). Foci of interstitial nephritis correlated less strongly ($P<0.02$). Foci of tubular regeneration did not correlate.

Discussion

The exact mechanisms involved in the progression of renal disease remain to be determined. It has been suggested (Brenner, 1985) that, following renal damage, adaptive processes that lead to increased glomerular capillary pressure, single nephron glomerular filtration rate and single nephron blood flow would lead to further nephron damage, thus creating an inexorable vicious cycle culminating in end-stage renal failure. Therefore, lowering glomerular capillary pressure would be expected to reduce glomerular injury and slow the progression of the disease. Other evidence indicates that angiotensin II mediates arterial and glomerular hypertension associated with renal damage. For example, angiotensin converting enzyme inhibition reduces intrarenal hypertension and glomerular hyperfiltration by preventing the intra-glomerular vascular actions of angiotensin II (Anderson *et al*., 1985; Brenner, 1985). It has also been suggested that factors other than glomerular hypertension may be important in the progression of chronic renal failure. Thus, glomerular hypertrophy (or hyperplasia) rather than glomerular hypertension has been suggested to be an important factor (Yoshida *et al*., 1988a,b). The protective effect of converting enzyme inhibition could, therefore, be due to inhibition of angiotensin II-induced mitogenic activity and/or fibrosis. The recognition of vasoactive substances such as angiotensin II and endothelin (King, 1995; Dubin *et al*., 1989) provided for a molecular basis for the proliferative nature of hypertensive target-organ damage. Evidence of nuclear activity, manifested as uptake of tritiated thymidine (Crane & Dutta, 1963) and hyperchromasia with mitotic activity (Campbell & Santos-Buch, 1966), were recognized as early hallmarks in the

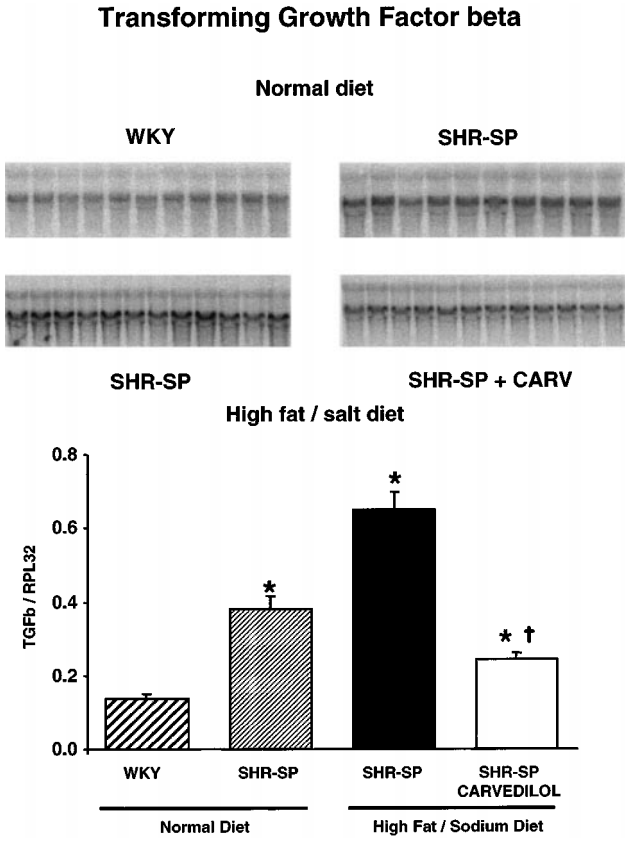

Figure 6 Effect of carvedilol on transforming growth factor β mRNA expression in WKY and SHR-SP rats fed a normal diet or high fat/sodium diet. Equal loading of gels was determined using expression of ribosomal protein L32 (rpL32). $n=5-6$ rats/group. $P<0.05$ versus WKY, †$P<0.05$ versus SHR-SP.

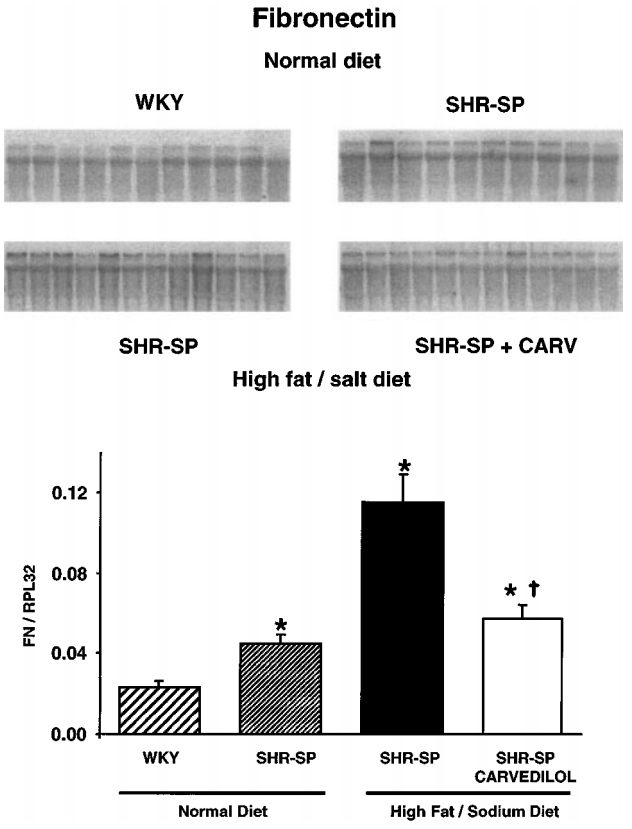

Figure 7 Effect of carvedilol on fibronectin mRNA expression in WKY and SHR-SP rats fed a normal diet or high fat/sodium diet. Equal loading of gels was determined using expression of ribosomal protein L32 (rpL32). $n=5-6$ rats/group. $P<0.05$ versus WKY, †$P<0.05$ versus SHR-SP.

histogenesis of arterial injury in experimental hypertension. Causative factors were suspected of having karyokinetic and karyorectic properties. Reduction of activity of the renin–angiotensin system by carvedilol likely is responsible at least in part for the attenuation of vascular damage induced by mitogenic and proliferative substances in this form hypertension induced in SHR-SP by high fat, high salt diet (Barone *et al*., 1996). Plasma renin activity was not measured in the present study, however, we have shown previously that carvedilol reduces this parameter in this model (Barone *et al*., 1996) and the 5/6 nephrectomized rat (Brooks *et al*., 1993).

It has become increasingly evident over the last few years that profibrotic factors such as TGFβ are also important in the progression of renal disease (Border *et al*., 1990). Evidence is growing that renoprotective agents such as inhibitors of the renin–angiotensin system not only have the ability to reduce the hypertension and associated progressive renal vascular disease, but also prevent increased expression of TGFβ and matrix proteins (Wong *et al*., 2000). Other evidence suggests that renal fibrosis associated with reduced blood flow is dependent on increased production of angiotensin II which, owing to its interactions with TGFβ, endothelin and PDGF-BB, is fibrogenic (Meyrier, 1999). It is of interest, therefore, that in the present study carvedilol provided significant renal protection, as evidenced by a reduction in proteinuria, an apparent improvement in renal function and histopathologic protection, without altering blood pressure. Indeed, carvedilol significantly reduced the levels of TGFβ, fibronectin, collagens I and III mRNA and fibrosis. It is possible that the renoprotective effects of carvedilol may also include a direct action on TGFβ-induced fibrosis independent of blood pressure.

It is important to note that carvedilol has diverse properties including α-adrenoceptor blockade, β1- and β2-adrenoceptor blockade and antioxidant activity. It also lowers PRA in this form of SHR-SP high fat, high salt diet hypertension. Which, if any of these properties, are important in the reduction of TGFβ expression is unclear. In studies where a direct comparison of carvedilol with propranolol has been conducted, carvedilol provided significantly greater protection than the pure beta blocker. For example, in 5/6 nephrectomized rats carvedilol, but not propranolol, caused a reduction in glomerulosclerosis despite similar antihypertensive responses (Peeters *et al*., 1995) and in hypertensive stroke-prone rats, carvedilol had a significantly greater benefit than propranolol (Barone *et al*., 1996). It is possible that the potent antiproliferative activity of carvedilol (Sung *et al*., 1993) may contribute to its therapeutic effect, since mesangial cell proliferation has been implicated in the progression of chronic renal disease and carvedilol has been shown to inhibit basal proliferation of glomerular mesangial cells as well as mesangial cell proliferation induced by epidermal growth factor and thrombin (Albrightson *et al*., 1992). Furthermore, carvedilol has a significantly greater

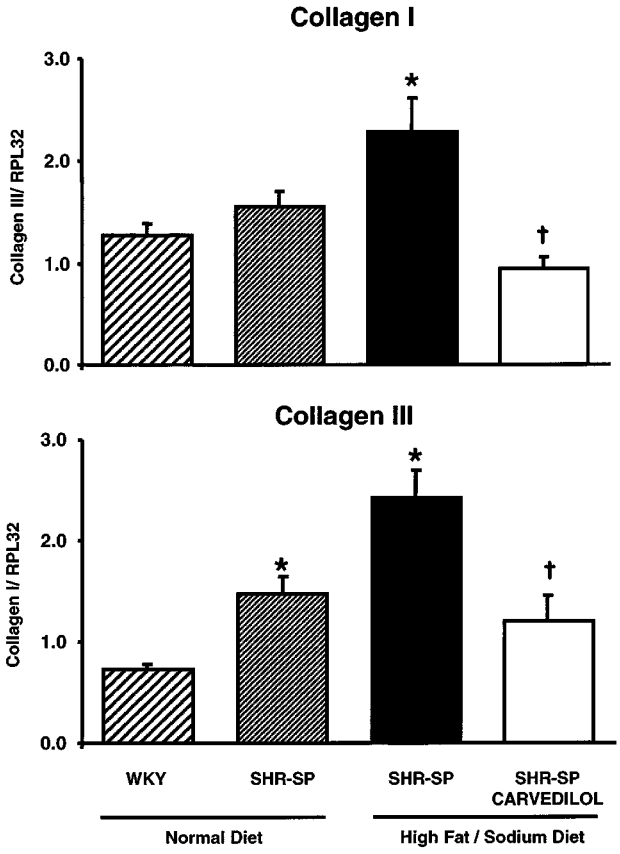

Figure 8 Effect of carvedilol on collagen I (upper panel) and collagen III (lower panel) mRNA in WKY and SHR-SP rats fed a normal diet or high fat/sodium diet as determined using quantitative polymerase chain reaction (Taqman). $n=5-6$ rats/group. $P<0.05$ versus WKY, †$P<0.05$ versus SHR-SP.

effect on epidermal growth factor and thrombin-induced proliferation than either celiprelol or propranolol (Albrightson *et al*., 1992).

It is also possible that carvedilol's potent antioxidant activity could contribute to its renoprotective effect because there is significantly higher production of hydrogen peroxide in damaged kidneys when compared to normal kidneys (Van den Branden *et al*., 1995). In studies with 5/6 nephrectomized rats, however, carvedilol had no effect on superoxide dismutase or glutathione peroxidase (Peeters *et al*., 1995). Others have made similar observations and have also demonstrated that carvedilol does not result in any change in renal hydrogen peroxide production or malonaldehyde concentrations in the renal tissue and/or urine of rats following renal damage (Van den Branden *et al*., 1995). It should be noted that one factor that does not appear to be involved in the renoprotective effects of carvedilol in this model is an alteration in lipid profiles. Previous studies indicate that carvedilol does not lower total cholesterol or triglicerides but slightly increases them in this model (Barone *et al*., 1996).

In this experiment, studies of renal function and tissue damage were carried out on matched set-sacrificed cohorts of rats studied after an initial phase in which 39 per cent of the SP-SFD high fat, high salt diet group had succumbed to accelerated hypertension in the first 11 weeks. The SP-SFD high fat, high salt diet controls and carvedilol-treated SP-SFD high fat, high salt diet matched pair-sacrificed rats from the initial phase demonstrated kidney crude scores of 2.57 ± 0.07 and 0.01 ± 0.01, respectively, in contrast to rats sacrificed between 12 and 14 weeks with scores of 1.74 ± 0.07 and 0.71 ± 0.33, respectively. Thus, the long surviving animals of the present report, in which no further fatalities occurred, suffered from a more insidious, slower, more chronically active process in which tissue remodelling and scarring were less marked than active renal vascular and nephron damage. These features of the experimental design may account for the relatively small increase in collagen I expression (hardly 2 fold) compared to fibronectin and collagen III expression (3 or more fold) and the relatively low level of histological evidence of fibrosis in the SP-SFD high fat, high salt rats with moderate renal injury in this report. In fact, renal collagen I mRNA expression tracted remarkedly close to foci of fibrosis considering that foci of recent pathologic fibrosis (scarring) may be metabolically more active than those of normal structural fibrosis.

In summary, our data indicate that carvedilol can provide significant renal protection in the absence of any antihypertensive activity. Thus, mechanisms other than blood-pressure-lowering appear to be involved in the renoprotective effects of carvedilol and this may involve reduced expression of profibrotic factors such as TGFβ1.

The authors are grateful to Ms Maria McDevitt for preparing this manuscript and to our colleagues in the Department of Laboratory Animal Sciences for providing outstanding animal care. We are also grateful to Jennifer Smith and Robert Santoianni of the Department of Pathology at the Emory University School of Medicine for excellent assistance with the histologic and photographic work.

References

ALBRIGHTSON, C.R., ZABKO-POTAPOVICH, B. & BROOKS, D.P. (1992). Effect of carvedilol on mitogen-stimulated mesangial cell proliferation. *Pharmacol. Commun.,* **1,** 267–272.

ANDERSON, S., MEYER, T.W., RENNKE, H.G. & BRENNER, B.M. (1985). Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *J. Clin. Invest.,* **76,** 612–619.

BARONE, F.C., NELSON, A.H., OHLSTEIN, E.H., WILLETTE, R.N., SEALEY, J.E., LARAGH, J.H. CAMPBELL, Jr W.G. & FEUERSTEIN, G.Z. (1996). Chronic carvedilol reduces mortality and renal damage in hypertensive stroke-prone rats. *J. Pharm. Exp. Ther.,* **279,** 948–955.

BORDER, W.A., OKUDA, S., LANGUINO, L.R., SPORN, M.B. & RUOSLAHTI, E. (1990). Suppression of experimental glomerulonephritis by antiserum against transforming growth factor-^{2}1. *Nature,* **346,** 371–374.

BRENNER, B.M. (1985). Nephron adaptation to renal injury or ablation. *Am. J. Physiol.,* **249,** F324–F337.

BROOKS, D.P., SHORT, B.G., CYRONAK, M.J., CONTINO, L.C., DICRISTO, M., WANG, Y.X. & RUFFOLO, Jr R.R. (1993). Comparison between carvedilol and captopril in rats with partial ablation-induced chronic renal failure. *Br. J. Pharmacol.,* **109,** 581–586.

CAMPBELL, JR W.G. & SANTOS-BUCH, C.A. (1966). Widely distributed necrotic arterial lesion induced in rabbits by experimental renal alterations. V. Light Microscopic studies of early lesions of arterial, casrdiac, and skeltal muscle. *Lab. Invest.,* **15,** 1856–1869.

CAMARGO, M.J.F., VON LUTTEROTTI, N., CAMPBELL, JR W.G., PECKER, M.S., JAMES, G.D., TIMMERMAN, P.B. & LARAGH, J.H. (1993). Control of blood pressure and end organ damage in maturing salt-loaded stroke-prone spontaneously hypertensive rats by oral angiotensin II receptor blockade. *J. Hyperten.,* **11,** 31–40.

CHOMCZYNSKI, P. & SACCHI, N. (1987). Single step method for RNA isolation by guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.,* **162,** 156–159.

CLELAND, J.G. (1998). Carvedilol for heart failure: more than just a beta-blocker? *Br. J. Hosp. Med.,* **58,** 493–497.

CRANE, W.A.J. & DUTTA, L.P. (1963). The utilisation of tritiated thymidine for the deoxyribonucleic acid systhesis by the lesions of experimental hypertension in rats. *J. Pathol. Bacterol.,* **86,** 83–92.

DAVIDSOHN, L. & HENRY, J.B. (1969). *Clinical Diagnosis by Laboratory Methods*. Philadelphia: W.B. Saunders.

DUBIN, D., PRATT, R.E., CROOKE, J.P. & DZAU, V.J. (1989). Endothelin, a potent vasconstrictor, is a vascular smooth muscle mitogen. *J. Vasc. Med. Biol.,* **1,** 150–154.

KING, A.J. (1995). Endothelins: Multifunctional Peptides with Potent Vasoactive Properties. 2nd edn. Chapter 38 in *Hypertension: Pathophysiology, Diagnosis and Management*, ed. Laragh, J.H. & Brenner, B.M. pp. 631–672. New York: Raven Press.

LEWIS, E.J., HUNSICKER, L.G., BAIN, R.P. & ROHDE, R.D. (1993). The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. *N. Engl. J. Med.,* **329,** 1456–1462.

LUNA, L.G. (1986). *Manual of histologic staining methods of the Armed Forces Institute of Pathology*. New York: McGraw-Hill.

MEYRIER, A. (1999). Vascular mechanisms of renal fibrosis. Vasculonephropathies and arterial hypertension. (Article in French). *Bull. Acad. Natl. Med.,* **183,** 33–45; discussion 45–56.

NAKAMOTO, H., SUZUKI, H., KATSUMATA, H., OHISHI, A., SARUTA, T. & SAKAGUCHI, H. (1988). Effects of carvedilol on renal function and blood pressure in three fifths nephrectomized spontaneously hypertensive rats loaded with high salt. *Drugs,* **36,** 160–164.

OKAMOTO, K., YAMORI, Y. & NAGAOHA, A. (1974). Establishment of stroke-prone spontaneously hypertensive rat (SHR). *Circ. Res.,* **34, 35** (Suppl.1): 143–153

OGIKU, N., SUMIKAWA, H., HASHIMOTO, Y. & ISHIDA, R. (1993). Prophylactic effect of imidapril on stroke in stroke-prone spontaneously hypertensive rats. *Stroke,* **24,** 245–252.

PACKER, M., BRISTOW, M.R., COHN, J.N., COLUCCI, W.S., FOWLER, M.B., GILBERT, E.M. & SHUSTERMAN, N.H. (1996). The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. *N. Engl. J. Med.,* **334,** 1349–1397.

PEETERS, P., VAN DEN BRANDEN, C., VANDEN HOUTE, K. & VERBEELEN, D. (1995). Effect of carvedilol (C) and propranolol (P) on glomerulosclerosis and antioxidant enzymes in 5/6 nephrectomy rats. *J. Am. Soc. Nephrol.,* **6,** 1027.

RODRIGUEZ-PEREZ, J.C., LOSADA, A., ANABITARTE, A., CABRERA-GALVAN, J., GARCIA, P., PALOP, L. & PLAZA, C. (1995). Effects of carvedilol, a new agent with multiple action on the preservation of renal structure and function in the rat remnant kidney. *J. Am. Soc. Nephrol.,* **6,** 684.

SUNG, C.P., ARLETH, A.J. & OHLSTEIN, E.H. (1993). Carvedilol inhibits vascular smooth muscle cell proliferation. *J. Cardiovasc. Pharmacol.,* **21,** 221–227.

VAN DEN BRANDEN, C., GABRIELS, M., VERELST, R., VANDEN HOUTE, K. & VERBEELEN, D. (1995). Decrease of focal glomerulosclerosis by carvedilol in rat remnant kidney is not associated with changed activities of antioxidant enzymes. *Cell Biol. Internatl.,* **19,** 720–721.

VOLPE, M., CAMARGO, M.J.F., MUELER, F.B., CAMPBELL, JR W.G., SEALEY, J.E., PECKER, M.S., SOSA, R.E. & LARAGH, J.H. (1990). Relation of plasma renin to end organ damage and to protection of K+ feeding in stroke-prone hypertensive rats. *Hypertension,* **15,** 318–326.

WANG, X., YUE, T.-L., BARONE, F.C. & FEUERSTEIN, G.Z. (1995). Demonstration of increased endothelial-leukocyte adhesion molecule-1 mRNA expression in rat ischemic cortex. *Stroke,* **26,** 1665–1669.

WONG, V.Y., LAPING, N.J., CONTINO, L.C., OLSON, B.A., GRYGIELKO, E. & BROOKS, D.P. (2000). Gene expression in rats with renal disease treated with the angiotensin II receptor antagonist, Eprosartan. *Physiological Genomics,* **42,** 35–42.

YOSHIDA, Y., FOGO, A. & SHIRAGA, H. (1988a). Serial micropuncture analysis of a single nephron function in the rat model of subtotal renal ablation. *Kidney Int.,* **33,** 855–867.

YOSHIDA, Y., FOGO, A., SHIRAGA, H., GLICK, A.D. & ICHIKAWA, I. (1988b). Serial micropuncture analysis of single nephron function in arresting progressive renal ablation. *Kidney Int.,* **33,** 855–867.

(Received June 25, 2001
Accepted August 7, 2001)