Nephropathy in Model Combining Genetic Hypertension With Experimental Diabetes

Enalapril Versus Hydralazine and Metoprolol Therapy

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We compared the effects of the angiotensin-converting enzyme inhibitor enalapril and a conventional antihypertensive regimen (hydralazine and metoprolol) on kidney function, albuminuria, and glomerular ultrastructure in hypertensive diabetic and nondiabetic rats. Diabetes was induced with streptozocin at 8 wk of age in spontaneously hypertensive (SHR) rats. Antihypertensive drugs were administered in drinking water from the time of induction of diabetes in all groups. Blood pressure reduction was equal in the diabetic and nondiabetic SHR rats receiving either enalapril or hydralazine plus metoprolol. In diabetic SHR rats, there was a rise in serum creatinine after 32 wk, which did not occur in diabetic rats treated with either antihypertensive regimen or in nondiabetic rats. Both drug regimens reduced albuminuria in diabetic and nondiabetic SHR rats to a similar degree. Enalapril and the combination of hydralazine and metoprolol were associated with decreased glomerular basement membrane thickness and glomerular volume in diabetic and nondiabetic SHR rats without significant effect on fractional mesangial volume. Thus, antihypertensive therapy retards the development of albuminuria, glomerular basement membrane thickening, and glomerular hypertrophy in the rat in the presence or absence of diabetes. No specific benefit of angiotensin-converting enzyme inhibition was observed in these hypertensive models of nephropathy. Human studies comparing the effects of different classes of antihypertensive drugs on kidney function, proteinuria, and glomerular morphology are warranted. *Diabetes* 39:1575–79, 1990

There has been recent interest in the capacity of angiotensin-converting enzyme (ACE) inhibitors to retard the progression of diabetic nephropathy (1). ACE inhibitors have been shown to decrease albuminuria in hypertensive insulin-dependent (type I) diabetic patients with established nephropathy (2) and normotensive type I diabetic patients with microalbuminuria (3). We previously described a model combining genetic hypertension with streptozocin-induced diabetes (STZ-D) in which diabetic spontaneously hypertensive (SHR) rats had increased albuminuria, glomerular basement membrane (GBM) thickness, glomerular volume, and a modest increase in serum creatinine compared with diabetic normotensive (Wistar-Kyoto) rats (4). In experimental diabetes, the ACE inhibitor enalapril has been shown to prevent the development of albuminuria and glomerulosclerosis in the STZ-D Munich-Wistar rat (5). In addition, we previously reported that enalapril therapy was associated with decreased GBM thickness without influencing mesangial expansion (6).

It has been suggested that ACE inhibitors may confer a specific benefit in ameliorating the progress of kidney disorders independent of their hypotensive action (7). This has been based on experimental studies comparing the effects of ACE inhibitors to other antihypertensive agents in the model of subtotal nephrectomy (7). In this study, the effects of the ACE inhibitor enalapril and the combination of hydralazine and metoprolol on kidney function, albuminuria, and glomerular ultrastructure were compared in diabetic and nondiabetic SHR rats over 32 wk.

RESEARCH DESIGN AND METHODS

Male SHR (Okamoto) rats derived from animals supplied by Y. Yamori in 1977 and bred in the Biological Research Laboratory at the Austin Hospital were used in this study. Rats weighing between 200 and 250 g and aged 8 wk were injected with 45 mg/kg body wt i.v. STZ (Boehringer Mannheim, Mannheim, FRG) after an overnight fast. Only animals with blood glucose levels >15 mM 1 wk after injection of STZ were included as diabetic animals in the study. Diabetic animals were randomized to receive either no treatment, 35

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mg/L enalapril (Merck, Rahway, NJ) in drinking water or a combination of 50 mg/L hydralazine (Ciba-Geigy, Basel) and 50 mg/L metoprolol tartrate (Ciba-Geigy) in drinking water. In nondiabetic SHR rats, animals were also randomized to the above three groups, but the doses of the drugs were doubled, because these animals had reduced water intake compared with diabetic rats. Because diabetic animals drink four- to fivefold more than nondiabetic animals, diabetic animals received approximately double the dose of either antihypertensive regimen.

All rats were caged in groups of four and fed a normal diet (GR 2+ rat cubes, Clarke King, Melbourne, Australia) containing 20% protein. At four weekly intervals over the 32-wk study, animals were weighed, and blood pressure was measured by indirect tail-cuff plethysmography in unanesthetized preheated rats (8). Before death, animals were individually housed in metabolic cages (Iffa Credo, L'Arbresele, France) for collection of 24-h urine samples for measurement of albuminuria. Daily urine volume was measured for each animal.

Serum glucose was measured by a glucose oxidase technique (9). Serum creatinine was measured by autoanalyzer (Beckman ASTRA, Palo Alto, CA). Urine was regularly tested for the presence of ketones (Ketostix, Ames, Mulgrave, Australia). Urinary albumin concentration was measured by a coated-tube radioimmunoassay after urine samples had been dialyzed for 48 h against running tap water followed by phosphate-buffered saline (pH 7.8) for a further 48 h (10). The interassay coefficient of variation was 10.6% (n = 12), and the lowest detection limit of the assay was 5 ng/ml.

Kidney histology was assessed by quantitative histomorphometry at the end of 32 wk. After the animals were anesthetized with pentobarbital sodium (Nembutal, Bomac Laboratories, Asquith, Australia), kidneys were perfused in vivo at arterial pressure via an intraaortic cannula, initially with saline, followed by 2.5% glutaraldehyde (11). The kidneys were fixed in formalin for light microscopy and then processed in paraffin. Two-micrometer sections were cut and stained with hematoxylin and eosin and periodic acid–Schiff. A separate portion was also prepared for electron microscopy. Briefly, 1-mm^3 cubes were cut from deep areas of the renal cortex as previously described (4). These sections were then left for a further 12 h in the glutaraldehyde fixative before insertion into 0.1 M phosphate buffer for 72 h. Sections were then dehydrated in acetone, followed by infusion with araldite-Epon resin. The block was then polymerized in an oven at 60°C for 3 days. Thick sections (1 μm) were cut and placed on a slide and stained with methylene blue. Where glomeruli were sighted by light microscopy, the blocks were trimmed down, and 50-mm sections were cut. Sections were picked up on a Formvar-coated single-hole grid and saturated with uranyl nitrate and Reynold's lead citrate. Sections were viewed under a JEOL 1200EX transmission electron microscope. Fifteen to twenty evenly spaced electron photomicrographs were obtained from two glomeruli/rat at a final magnification between ×15,000 and ×18,000. With each set of electron micrographs, a calibration grid was photographed to monitor the exact magnification. GBM thickness was measured by the orthogonal-intercept method (12). Glomerular volume was measured by light microscopy with a point-counting method in which a minimum of 60 glomeruli were assessed per rat (13). Mesangial expansion was assessed by calculation of the fractional mesangial volume as previously described (14). Mesangium was identified in electron micrographs by an independent observer (B.E.C.) and assessed by a point-counting method with a 180-point grid to determine the percentage of glomerular tuft occupied by mesangium.

Urinary albumin excretion data were analyzed after logarithmic transformation. Kidney weight data are shown as the mean of the left and right kidneys. Comparisons of normally distributed variables between different groups over the study period were performed by analysis of variance with or without repeated measures with the Statview SE and Graphics program (Brainpower, Calabasas, CA) on a Macintosh SE personal computer (Apple, Cupertino, CA). Comparisons between group means were performed by Fisher's least-significant difference test (15).

RESULTS

Weight, glycemic control, urine volume, kidney function, and kidney weight are shown in Table 1. Weight gain was reduced in diabetic rats compared with nondiabetic SHR rats ($P < 0.001$). Antihypertensive therapy did not influence body weight in any of the diabetic groups. In nondiabetic rats treated with hydralazine and metoprolol (HM), body weight was significantly increased compared with the other nondiabetic groups. There was no significant difference in the degree of hyperglycemia between untreated, enalapril-treated, or HM-treated diabetic SHR rats. Ketonuria was not

TABLE 1
Clinical characteristics of untreated rats and rats treated with enalapril or hydralazine and metoprolol

	n	Weight (g)	Blood pressure (mmHg)	Urine volume (ml/24 h)	Serum glucose (mM)	Serum creatinine (μM)	Kidney weight (g)
Nondiabetic	14	429 ± 6	215 ± 6	14 ± 1	7.1 ± 0.7	46 ± 2	2.48 ± 0.06
+ Enalapril	13	448 ± 10	174 ± 4*	18 ± 2	7.6 ± 0.4	48 ± 3	2.34 ± 0.07
+ Hydralazine and metoprolol	12	501 ± 22*†	174 ± 5*	15 ± 2	5.8 ± 0.3†	43 ± 2	2.34 ± 0.07
Diabetic	11	303 ± 19	200 ± 3	81 ± 6	32.5 ± 3.7	81 ± 9	2.82 ± 0.09
+ Enalapril	16	302 ± 7	154 ± 4*	79 ± 4	30.2 ± 1.5	43 ± 3*	2.59 ± 0.16
+ Hydralazine and metoprolol	14	270 ± 14	155 ± 2*	81 ± 12	29.5 ± 1.5	37 ± 4*	2.39 ± 0.22

Values are means ± SE for data at 32 wk of study.
*$P < 0.01$ vs. untreated diabetic or nondiabetic.
†$P < 0.01$ vs. nondiabetic + enalapril.

detected in any urine samples from the diabetic animals. There was an increase in urine volume in diabetic rats compared with nondiabetic SHR rats ($F = 288$, $P < 0.001$). Drug therapy did not influence urine volume.

Although no groups developed overt kidney failure, there was a significant rise of ~80% in serum creatinine in diabetic SHR rats, which was not seen in nondiabetic SHR rats. The rise in serum creatinine observed in diabetic SHR rats was prevented by either enalapril or HM treatment. There was a modest increase in kidney weight in diabetic rats compared with nondiabetic SHR rats ($P = 0.03$). Treatment did not significantly influence kidney weight.

There was no significant difference in blood pressure between diabetic and nondiabetic SHR rats. Enalapril and HM significantly reduced blood pressure in diabetic and nondiabetic SHR rats (Table 1). There was no significant difference in mean systolic blood pressure over the study period between enalapril- and HM-administered diabetic SHR rats (Fig. 1). In nondiabetic SHR rats, both regimens also significantly reduced blood pressure ($P < 0.01$), but to a lesser extent than observed in the diabetic animals. This may be due to the reduced fluid intake in the nondiabetic animals, resulting in the animals receiving a lower dose of each antihypertensive drug.

Both enalapril and HM therapy retarded the development of albuminuria in diabetic and nondiabetic SHR rats (Fig. 2). In particular, there was no significant difference between the two hypotensive regimens on albuminuria in diabetic or nondiabetic SHR rats.

Diabetic SHR rats had increased GBM thickness compared with nondiabetic SHR rats ($F = 119.9$, $P < 0.001$; Fig. 2). In diabetic SHR rats, GBM thickness was reduced in enalapril- and HM-administered rats compared with untreated rats. A similar reduction in GBM thickness was observed in nondiabetic SHR rats with administration of either hypotensive regimen. There was no significant difference between diabetic and nondiabetic rats with respect to the effect of the two therapeutic regimens in retarding GBM thickening ($F = 2.3$, $P = 0.14$).

Diabetic SHR rats had a modest increase in fractional mesangial volume compared with nondiabetic SHR rats ($F = 5.7$, $P = 0.02$; Fig. 2). However, in diabetic and nondiabetic SHR rats, neither drug influenced fractional mesangial volume.

There was no difference in glomerular volume between diabetic and nondiabetic SHR rats (Fig. 2). Glomerular volume was reduced by antihypertensive agents in diabetic and nondiabetic SHR rats ($F = 8.8$, $P < 0.001$). The effects of enalapril and HM therapy on glomerular volume were similar. The effects of treatment were similar in diabetic and nondiabetic SHR rats ($F = 0.16$, NS).

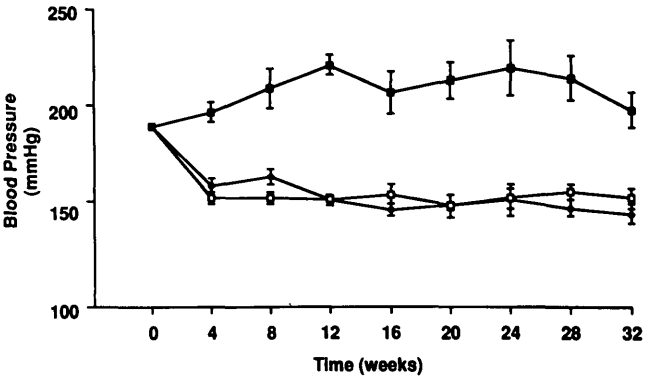

FIG. 1. Blood pressure in diabetic and nondiabetic spontaneously hypertensive (SHR) rats. Systolic blood pressure measurements are shown as means ± SE over 32 wk in untreated (■), enalapril-treated (♦), and hydralazine plus metoprolol–treated (□) diabetic SHR rats.

DISCUSSION

This study showed that antihypertensive therapy ameliorates both functional and structural parameters of kidney damage in hypertensive rats in the absence or presence of diabetes. However, no specific advantage of the ACE inhibitor enalapril over the combination HM regimen on glomerular injury was detected in diabetic or nondiabetic SHR rats. Both hypotensive regimens prevented the rise in serum creatinine in diabetic SHR rats and decreased albuminuria, GBM thickness, and glomerular hypertrophy without influencing mesangial expansion in diabetic and nondiabetic SHR rats. Note that the amelioration of glomerular damage as assessed by albuminuria and glomerular ultrastructural parameters was observed not only in diabetic but also in nondiabetic hypertensive animals. These results indicate that systemic hypertension is a major determinant of glomerular injury in diabetes and that many of the functional and structural characteristics that have been attributed to diabetes, e.g., albuminuria and GBM thickening, may be due in part to hypertension.

It has been suggested that ACE inhibitors may have a specific benefit in retarding glomerular damage, because these agents may have a specific intrarenal hemodynamic action in reducing intraglomerular pressure via reduction in angiotensin II (ANG II)-mediated efferent arteriolar vasoconstriction (16). However, recent studies suggested that the specificity of these agents on intraglomerular hemodynamics may not be as clear-cut as previously considered. In the uninephrectomized SHR model, triple therapy (reserpine, hydralazine, and hydrochlorothiazide) was equipotent to ACE inhibitor therapy in reducing glomerular capillary hydraulic pressure, reducing proteinuria, and decreasing glomerular injury (17). In the subtotal-nephrectomy model, studies have suggested that ACE inhibitors may confer a specific benefit over either triple therapy (7) or the Ca^{2+} antagonist felodipine (18) in retarding the development of proteinuria and glomerulosclerosis. However, in a more recent study in rats after subtotal nephrectomy, triple therapy was equipotent to enalapril therapy in preserving glomerular structure (19). This renoprotective effect occurred despite triple therapy failing to reduce intraglomerular pressure.

It has been suggested that the diabetic kidney may be particularly susceptible to the damaging effects of hypertension, because experimental diabetes has been shown to be a state of afferent vasodilation, thereby permitting direct transmission of systemic blood pressure to the glomerular microcirculation (1,20). A recent study observed that non–insulin-treated diabetic rats had a reduced capacity to autoregulate kidney blood flow compared with insulin-treated moderately hyperglycemic nondiabetic rats (21). Micropuncture studies in diabetic SHR rats have shown that blood pressure treatment was effective in reducing intraglomerular

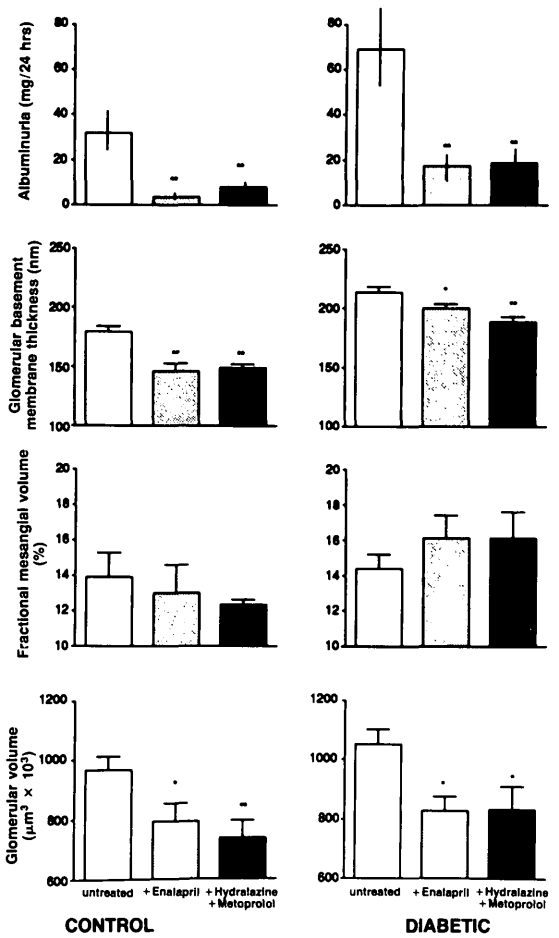

FIG. 2. Albuminuria, glomerular basement membrane thickness, fractional mesangial volume, and glomerular volume for control versus diabetic spontaneously hypertensive rats. Values are means ± SE except for albuminuria data, which are shown as geometric means and tolerance factors. *$P < 0.025$, **$P < 0.01$, vs. untreated.

and systemic pressure (22). Similar effects of antihypertensive treatment on intraglomerular pressure have been observed in the normotensive diabetic animals studied by Anderson et al. (20). Therefore, it is conceivable that, in the setting of afferent arteriolar vasodilation as observed in experimental diabetes, the reduction in systemic blood pressure by any antihypertensive agent would result in a direct reduction in intraglomerular pressure. This would explain the lack of a specific advantage of ACE inhibitors in preventing glomerular injury in this study.

In normotensive diabetic rats, enalapril therapy has been shown to prevent the development of albuminuria, reduce glomerulosclerosis, and retard GBM thickening (5,6). In a recent study in normotensive Munich-Wistar diabetic rats, captopril appeared to be more effective than triple therapy in preventing glomerular damage (21). The advantage of ACE inhibition observed in that study was not observed in this study. There are several possible factors in our study that could explain the differences in results from those observed by Anderson et al. (20). In this study, the animals were hypertensive, hyperglycemia was not treated with insulin, and rather than triple therapy, the conventional antihypertensive regimen included the β-blocker metoprolol, which may itself suppress the renin-angiotensin system (23).

The lack of an effect on fractional mesangial volume by either hypotensive regimen is consistent with our previous report on the effects of enalapril therapy in hypertensive and diabetic rats (6). It is possible that metabolic control is the major determinant of mesangial expansion. Neither antihypertensive treatment influenced plasma glucose levels, which could explain the lack of an effect on fractional mesangial volume. In previous studies in which excellent glycemic control was achieved in diabetic rats with either insulin therapy (24) or pancreatic islet transplantation (25), there was a reduction of mesangial volume. In the non–insulin-treated diabetic rat, mesangial expansion occurs despite reduced intraglomerular pressure (26), which is consistent with the hypothesis that intrarenal hemodynamic factors may not be the major determinants of fractional mesangial volume. It is also possible that the antihypertensive agents in this study did not modify glomerular flow and that glomerular flow rather than pressure may be the important hemodynamic parameter associated with mesangial expansion. This would explain the failure of pancreatic transplantation to ameliorate mesangial expansion in the uninephrectomized diabetic rat (27).

In this study, glomerular hypertrophy, which had been previously reported by our group in SHR rats (4), was reduced by both hypotensive regimens. It has recently been suggested that glomerular hypertrophy rather than kidney hemodynamic factors may be more closely linked to ultimate glomerular damage (28). The cause of glomerular hypertrophy in SHR rats is unknown, but the efficacy of both hypotensive regimens in reducing glomerular volume observed in this study suggests that blood pressure may be an important determinant of glomerular size. In the recent study of subtotal nephrectomy by Yoshida et al. (19), triple therapy and ACE inhibition also reduced glomerular hypertrophy. Similar equivalent reductions in glomerular size with either the ACE inhibitor or the triple-therapy regimen have also been reported in the study of diabetic rats by Anderson et al. (20). A possible mechanism for the reduction of glomerular size by either regimen could relate to suppression of intrarenal ANG II either by the ACE inhibitor enalapril or the β-blocker metoprolol. Recently, ANG II has been reported to be an important trophic factor acting via induction of genes associated with the cellular growth response (29). Therefore, suppression of intrarenal ANG II levels could explain not only the reduction in glomerular volume but also the retardation of GBM thickening, which was observed in this study. In a preliminary report, enalapril has been shown to attenuate the maturational growth of glomeruli in young rats (30). The mechanism by which antihypertensive drugs retard glomerular hypertrophy is not well understood; it remains to be shown whether it is mediated by ANG II. A study has suggested that antihypertensive drugs including captopril and hydralazine may inhibit hypertrophy by preventing increases in DNA synthesis (31).

There have been no long-term studies comparing the effects of ACE inhibitors to conventional antihypertensive agents on kidney function and albuminuria in hypertensive diabetic patients. However, a study of the effect of the ACE inhibitor captopril on decline in kidney function and urinary albumin excretion (2) revealed similar beneficial effects to those observed in a previous study by the same group with

hydralazine, β-blockers, and diuretic treatment (32). In a previous study in normotensive subjects, enalapril therapy reduced albuminuria over 12 mo (3). No long-term comparisons between different classes of hypotensive agents have been published. A study over the relatively short period of 6 wk suggested a possible advantage of the ACE inhibitor captopril over nifedipine in reducing albuminuria in normotensive type I diabetic patients (33). In contrast, a preliminary report of a 3-mo study suggested no specific benefit of ACE inhibitors in a study comparing the ACE inhibitor perindopril to nifedipine in microalbuminuric diabetic subjects (34). The results of this experimental study indicate that longer-term studies comparing the effect of ACE inhibitors to other hypotensive agents on the progression of diabetic kidney disease are warranted in human diabetic subjects.

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