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LABORATORY INVESTIGATION

Long-term effects of antihypertensive regimens on renal hemodynamics and proteinuria

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Long-term effects of antihypertensive regimens on renal hemodynamics and proteinuria. The long-term effects of different antihypertensive regimens were studied in uninephrectomized beagles with alloxan-induced diabetes mellitus. Mean arterial pressure (MAP) was elevated ($P < 0.05$) in untreated diabetic dogs. Treatment of diabetic dogs with an angiotensin converting enzyme inhibitor (ACEI; lisinopril), a calcium antagonist (CA; TA-3090), or both lowered MAP. At one year, the RBF, GFR, and SNGFR were similarly elevated ($P < 0.05$) in all groups of diabetic dogs. The increase in SNGFR present in untreated diabetic dogs was primarily attributable to an increased ($P < 0.05$) glomerular capillary pressure (P_{GC}). Treatment with lisinopril lowered the P_{GC} to a mean value that was indistinguishable from that for nondiabetic dogs. In contrast, diabetic dogs treated with TA-3090 had an elevated P_{GC}. While untreated diabetic dogs exhibited marked increases in glomerular volume ($P < 0.05$ vs. nondiabetic dogs), treatment with lisinopril and TA-3090, either alone or in combination, blunted the extent of glomerular hypertrophy observed in diabetic dogs ($P < 0.05$ vs. untreated diabetic dogs). Proteinuria was similarly reduced ($P < 0.05$ vs. untreated diabetic dogs) in dogs treated with lisinopril and TA-3090. Combination therapy of diabetic dogs produced a further significant ($P < 0.05$) decrement in proteinuria. We conclude that although treatment of diabetic dogs with either lisinopril or TA-3090 results in differential effects on P_{GC}; each produces a similar decrement in proteinuria. Further, combination therapy has a greater effect on proteinuria than either agent alone. These data suggest that CA and ACEI have different effects on renal hemodynamics in diabetic dogs and, further, that both glomerular hypertension and glomerular hypertrophy are involved in the proteinuria observed in diabetic dogs.

Progressive glomerulopathy associated with proteinuria and systemic hypertension is a leading cause of renal failure and death in diabetic human beings [1–5]. It has been proposed that changes in glomerular structure and function observed in diabetic individuals are responsible for the genesis and/or progression of renal injury [2–4, 6]. This hypothesis is supported by studies with rodent models of diabetic renal disease in which elevations of glomerular capillary pressure (P_{GC}) and/or glomerular volume have been causally associated with progressive destruction of renal tissue [7–12].

Several studies demonstrate that angiotensin-converting enzyme inhibitors (ACEI) preserve glomerular structure and function in rodent models of diabetes [8–11]. The administration of ACEI to rats with streptozotocin-induced diabetes consistently lowers P_{GC} and albuminuria [8, 9, 11]. The use of ACEI may also attenuate glomerular hypertrophy in diabetic rats [9], although this effect has not been consistently observed [11]. Conversely, studies with calcium antagonists (CA) have demonstrated variable results in rats [10–15]. In uninephrectomized rats with streptozotocin-induced diabetes, the dihydropyridine-type CA nifedipine lowered arterial pressure but failed to reduce glomerular pressure and albuminuria, and did not prevent glomerulosclerosis [11]. The absence of effect on glomerulosclerosis and albuminuria has been noted with other dihydropyridine-type CA [12, 13]. Studies with papaverine- or benzothiaprine-type CA demonstrate variable effects on renal hemodynamics and/or proteinuria in diabetic rats [10, 14, 15]. Thus the relative ability of CA to attenuate progression of diabetic renal disease is inconsistent and may vary with the type of CA used.

Although little is known about glomerular hemodynamics in nonrodent animals with diabetes, changes in renal hemodynamics observed in human beings with Type I diabetes parallel those observed in rodent models of diabetes [3, 6, 16–19]. Overt diabetic nephropathy in people is preceded by changes in renal structure and function that include elevations of whole kidney GFR [16–18] and glomerular size [17, 18]. While effective antihypertensive therapy may slow the rate of decline in GFR in affected people [20, 21], the mechanism of this protection is not well understood. In particular, the changes in glomerular hemodynamics that occur in diabetic people and the effects of antihypertensive therapy on these adaptations remain incompletely characterized. While ACEI consistently lower proteinuria in diabetic people [20–22], studies with dihydropyridine-type CA indicate no effect or an increase in protein loss [23–25]. In contrast, studies with the benzothiaprine-type CA diltiazem [24, 26, 27] or the use of the papaverine-type CA verapamil alone or in combination with an ACEI [21] demonstrate a reduction of proteinuria.

Glomerular structural changes were demonstrated in diabetic beagles studied for one year after diabetes mellitus was induced by alloxan administration [28]. However, experimental studies of the long-term effects of diabetes mellitus on renal function in dogs have not been reported. Consequently, the effects of diabetes on canine renal and glomerular function and the hemodynamic effects of antihypertensive agents in this setting

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remain poorly characterized. The purpose of the current study was to compare the effects of the chronic administration of an ACEI (lisinopril) and a benzothiaprine CA (TA-3090), alone and combined, on renal hemodynamics, glomerular volume, and proteinuria in diabetic beagles.

Methods

General (Groups I to V)

Experiments were performed on 29 adult beagle dogs of either sex weighing 13.0 ± 0.3 kg. All dogs underwent right nephrectomy. Twenty-three of these dogs received an intravenous infusion of 60 mg/kg of alloxan to induce diabetes mellitus [28]. The left renal artery was occluded during and for five minutes after the infusion of alloxan to prevent acute tubular necrosis. Control dogs (Group I) were studied in a manner similar to the other groups. Dogs with diabetes mellitus were randomly assigned to insulin therapy alone (Group II; $N = 8$), insulin therapy plus daily oral administration of the ACEI lisinopril (Group III; $N = 6$), insulin therapy plus daily oral administration of the CA TA-3090 (Group IV; $N = 5$), or insulin therapy plus combined daily oral administration of lisinopril and TA-3090 (Group V; $N = 4$). The goal of insulin therapy was to achieve a plasma glucose concentration in the range of 350 to 450 mg/dl immediately prior to the subsequent insulin dosage, with adjustments of insulin dosage if results of several consecutive blood glucose determinations fell outside of this range. All dogs were subsequently fed a diet which contained 15.4% protein and 0.21% sodium (Hill's Pet Products, Topeka, Kansas, USA). Quantities were adjusted to maintain stable body weights. Plasma creatinine was assessed monthly. MAP was measured in conscious dogs by direct femoral arterial puncture at monthly intervals in treated dogs and every two months in Group I. Dosages of antihypertensive agents were adjusted to maintain MAP between 95 and 105 mm Hg.

Proteinuria and endogenous creatinine clearance (Groups I to V)

Every two months during the study, the dogs were subjected to 24-hour metabolism cage collections to quantify the degree of proteinuria, and 24-hour endogenous creatinine clearance was determined at four month intervals. Dogs were fed and administered their usual medications during these collections.

Renal hemodynamic studies (Groups I–IV)

Prior to these studies, the dogs were fasted for 16 to 20 hours. The dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and were prepared for micropuncture and renal clearance studies as previously described [29–31]. The trachea was intubated and respiration was regulated mechanically (Harvard Apparatus, South Natick, Massachusetts, USA). A catheter was placed in the left jugular vein. A solution containing 0.9% saline and 2.5% inulin was infused at a rate sufficient to maintain a plasma inulin concentration of approximately 0.8 mg/ml in all dogs. Diabetic dogs received additional 0.9% saline solution to replace excessive urinary losses during the procedure. Arterial pressure was measured through a catheter inserted into the femoral artery. The catheter was connected to a Statham pressure transducer (Statham Laboratories, Hato Rey, Puerto Rico). Output was recorded on a Grass Polygraph (Grass Instruments, Quincy, Massachusetts, USA). Blood samples were also collected through this catheter.

The left kidney was exposed through a flank incision, and the proximal renal artery and vein and the ureter were dissected free of adjacent tissue. An electromagnetic flow transducer was placed around the renal artery and connected to a square-wave flowmeter (Carolina Medical Electronics, King, North Carolina, USA) to measure renal blood flow (RBF). The ureter was catheterized to allow timed urine collections.

An adjustable clamp was placed around the renal artery distal to the flow probe or around the aorta proximal to the renal artery in dogs with a short renal artery. A curved 23-gage needle was placed in the renal artery distal to the lucite clamp and connected to a Statham pressure transducer (Statham Laboratories) for measurement of renal arterial pressure (RAP). Adjustment of the vascular clamp allowed graded reductions in RAP. The renal arterial line was infused with 0.2 ml/min heparinized, isotonic saline solution to maintain patency. The left kidney was then placed on a Lucite holder and prepared for micropuncture by removal of approximately 3 cm^2 of the renal capsule. This area was continuously bathed with warm (39°C), heparinized, isotonic saline solution dripped through a quartz rod, which was used to illuminate the micropuncture field. Flexible tape was placed around the micropuncture field to assist in stabilization. An agar well was placed around the micropuncture field to maintain an isotonic saline solution pool. The remainder of the kidney was covered with warm, saline-soaked gauze and loosely wrapped with plastic wrap.

Forty-five minutes later, micropuncture collections and micropressure measurements were made at spontaneous RAP. The MAP, RAP, and RBF were continuously monitored, and two or three timed ureteral collections of 15 to 20 minutes each were made during these micropuncture studies for determination of inulin clearance (GFR). A blood sample was collected at the midpoint of each timed urine collection. For SNGFR determination, a sharpened pipette (12 to 18 μm tip) was filled with Sudan black-stained castor oil. The pipette tip was inserted into a proximal tubule, and an oil column of at least 5 tubule diameters was inserted. Gentle aspiration was applied to initiate the collection and to maintain the oil block in a constant position. Timing was started at the initiation of collection and continued for one to two minutes. After removal, the pipette was stored in mineral oil prior to determining the volume and inulin concentration of the collection. Hydraulic pressures were determined by a micropressure servo-null system (Instrumentation for Physiology and Medicine, San Diego, California, USA). At least three free-flow proximal tubular pressures (PTP), stop-flow proximal tubule pressures (SFP), and peritubular capillary pressures (PCP) were measured for each dog. The PCP measurements were taken from the earliest accessible site on the cortical surface of the kidney.

Autoregulation studies (Groups I to IV)

Following clearance and micropuncture studies at spontaneous renal perfusion pressure, RAP was reduced to approximately 100 mm Hg and measurements were repeated. At the end of each experiment, the electromagnetic flow probe was calibrated *in situ* by the placement of a catheter directly into the renal artery and collection of timed blood samples into a graduated cylinder.

Glomerular volume determinations (Groups I to V)

Formalin fixed tissue was processed by routine histologic methods, and sections were stained with periodic acid-Schiff and hematoxylin dyes. Planar area of 50 outer cortical glomerular tufts, which were chosen randomly, were measured in these sections with a planar morphometry image analysis system (Southern Micro Instruments, Atlanta, Georgia, USA) to determine glomerular volume as previously described [30]. The morphometry system was calibrated with a micrometer reticle (Reichert Scientific Instruments, Buffalo, New York, USA).

Analyses and calculations

Inulin concentration in both ureteral urine and plasma was measured by an anthrone colorimetric technique [30]. Whole kidney GFR was calculated by the standard clearance formula. Microhematocrit (HCT) measurements were performed on all arterial blood samples. Renal plasma flow (RPF) was derived from the measured values for RBF and HCT. Whole kidney filtration fraction (FF) was determined from GFR and RPF. Measurements of sodium and potassium concentrations (Instrumentation Laboratory, Lexington, Massachusetts, USA) and osmolality (Wescor, Logan, Utah, USA) were performed on all urine and plasma samples. Plasma colloid osmotic pressure was measured with a membrane osmometer (Wescor). Urine protein concentration was determined by the Coomassie brilliant blue technique [32].

Monthly glucose determinations and plasma and urine concentrations of creatinine were determined by a semi-automated method (Beckman Instruments, Fullerton, California, USA). Blood glucose concentration was assessed at least once weekly with the use of reagent strips read by a semi-automated method (Becton-Dickinson, Franklin Lakes, New Jersey, USA). Total plasma glycosylated hemoglobin concentration was determined by ion exchange chromatography (Isolab Incorporated, Akron, Ohio, USA).

Tubular fluid inulin concentration was determined in duplicate by a ultramicrofluorometric method [29]. The SNGFR was taken as the product of volume flow rate, determined with the aid of constant bore tubing [29], and the tubular fluid-to-plasma inulin ratio. Glomerular blood flow (GBF) and glomerular plasma flow (GPF) were computed from the filtration fraction (FF), single nephron glomerular filtration rate, and hematocrit:

$$GBF = SNGFR/[FF(1 - HCT)]$$

$$GPF = SNGFR/FF$$

Whole kidney FF values were used for these calculations since efferent arteriolar blood samples could not be obtained routinely during the study. Glomerular capillary pressure was estimated from the sum of SFP and plasma colloid osmotic pressure (πa). Single nephron vascular resistances, preglomerular (R_A), efferent arteriolar (R_E), and total (R_T) were estimated by the expressions:

$$R_A = (MAP - P_{GC})/GBF$$

$$R_E = (P_{GC} - PCP)/(GBF - SNGFR)$$

$$R_T = MAP/GBF$$

The glomerular ultrafiltration coefficient, K_f, was calculated using the integrated solution to the general differential equation described previously [29–31],

$$K_f = (SNGFR/\Delta P) \times [1 - A \times \ln(1 - B)]$$

where

$$A = (R \times \pi a)/[FF \times \Delta P \times (R + \pi a)]$$

and

$$B = [FF \times \Delta P \times (R + \pi a)]/[R \times (\Delta P - \pi a)].$$

In these equations, ΔP is the glomerular transcapillary hydraulic pressure gradient, πa is the afferent colloid osmotic pressure, and R is a constant that relates πa to FF and efferent colloid osmotic pressure (πe). This approach is particularly useful in the dog because the value of R (43) is affected only slightly by variations in albumin-to-globulin ratios, which are substantial in this species [29].

Following measurements at reduced renal perfusion pressure, values for RBF obtained at spontaneous and reduced renal perfusion pressure were compared. This comparison allowed the determination of a renal autoregulatory index for RBF for each dog, which was taken as the ratio of the percent change in GFR divided by percent change in MAP. Autoregulatory indices for GFR were calculated in a similar manner.

Statistical analyses

Values are reported as means ± SEM. Data were compared among groups by analysis of variance. Where a significant effect was identified, group means were compared by the Fisher's PLSD comparison test. A P value <0.05 was considered indicative of a statistically significant difference.

Results

General (Groups I to V)

A comparable degree of hyperglycemia was present in all groups of diabetic dogs (Fig. 1). Few changes in insulin dosage were necessary after the first two months of the study, and the mean insulin dosages were similar in the four diabetic groups, averaging 0.9 ± 0.1 U/kg in Group II, 0.9 ± 0.1 U/kg in Group III, 1.0 ± 0.1 U/kg in Group IV, and 0.9 ± 0.2 U/kg in Group V. Glycosylated hemoglobin values were similarly elevated in all groups of diabetic dogs (Table 1). For the final month of the study, mean daily food intake values for Group II (34.5 ± 1.4 g/kg body wt/day), Group III (30.7 ± 4.3 g/kg body wt/day), Group IV (33.1 ± 2.9 g/kg body wt/day), and Group V (34.8 ± 5.1 g/kg body wt/day) were not different. However, the food intake for all groups of diabetic dogs exceeded the corresponding value for Group I (21.0 ± 1.3 g/kg body wt/day). There were no significant differences in body weight among groups (Table 1).

Values for MAP in awake dogs (Fig. 2) indicated the presence of mild systemic hypertension in the untreated diabetic dogs (Group II) and a similar, modest reduction of MAP in all treated groups (Groups III, IV, and V). At the time of renal hemodynamic studies, directly measured MAP was significantly elevated in Group II only. During the study, dosages of the

Table 1. Systemic and renal parameters in dogs 1 year after the induction of diabetes mellitus

Group	N	Body weight kg	Hb A_1 %	S_{Cr} mg/dl	BUN mg/dl	Hct %	TP g/dl
Group I	6	13.2 ± 0.7	6.9 ± 0.3	1.3 ± 0.1	13.3 ± 0.9	39.7 ± 2.6	5.9 ± 0.2
Group II	8	13.6 ± 0.5	9.8 ± 0.4[a]	0.7 ± 0.1[a]	13.4 ± 1.5	43.0 ± 1.8	6.5 ± 0.3
Group III	6	14.0 ± 0.8	9.6 ± 0.5[a]	0.8 ± 0.1[a]	14.8 ± 1.5	40.7 ± 1.7	6.3 ± 0.3
Group IV	5	12.9 ± 1.4	9.7 ± 1.0[a]	0.9 ± 0.1[a]	12.6 ± 3.0	40.8 ± 1.2	6.8 ± 0.5
Group V	4	12.3 ± 0.6	9.5 ± 1.0[a]	0.9 ± 0.1[a]	11.0 ± 1.3	41.2 ± 2.5	6.2 ± 0.3

Values are means ± SEM. Abbreviations are: Hb A_1, total glycosylated hemoglobins; S_{Cr}, serum creatinine; BUN, blood urea nitrogen; Hct, hematocrit; TP, arterial plasma total protein content.
[a] $P < 0.05$ vs. Group I

Table 2. Results of renal clearance studies 1 year after the induction of diabetes mellitus in dogs

Group	N	Plasma glucose mg/dl	MAP mm Hg	RBF ml/min/kg body wt	GFR ml/min/kg body wt	FF %	UV ml/min
Group I	6	96 ± 2	116.0 ± 5.7	11.5 ± 0.8	1.90 ± 0.04	28 ± 2	0.3 ± 0.1
Group II	8	388 ± 28[a]	126.0 ± 6.9	16.8 ± 1.6[a]	3.20 ± 0.37[a]	33 ± 1[a]	0.9 ± 0.2[a]
Group III	6	379 ± 21[a]	119.5 ± 6.7	18.0 ± 2.1[a]	2.86 ± 0.36[a]	27 ± 1[b]	1.1 ± 0.1[a]
Group IV	5	364 ± 554[a]	116.2 ± 5.3	17.3 ± 2.6[a]	3.07 ± 0.31[a]	31 ± 3	1.2 ± 0.3[a]

Values are means ± SEM. Abbreviations are: MAP, mean arterial pressure; RBF, renal blood flow; GFR, glomerular filtration rate; FF, filtration fraction; UV, urine flow.
[a] $P < 0.05$ vs. Group I
[b] $P < 0.05$ vs. Group II

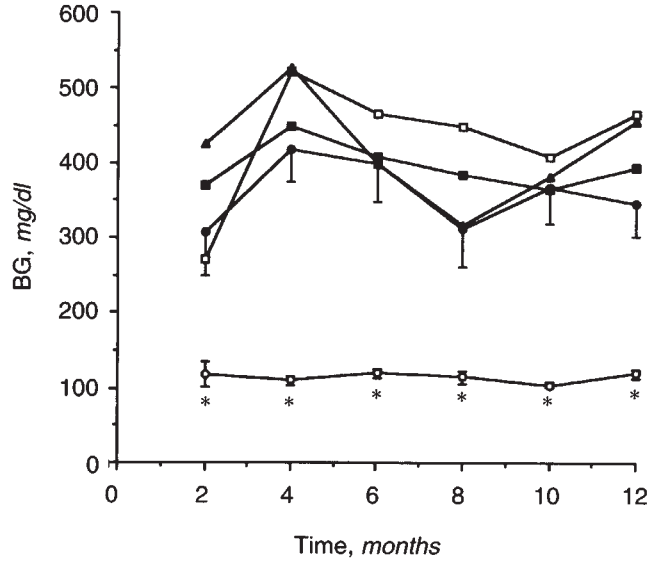

Fig. 1. *Serial values for blood glucose (BG) measurements in dogs.* Serial values for BG in control dogs (○, Group I, $N = 6$), untreated diabetic dogs (●, Group II, $N = 8$), or diabetic dogs treated with lisinopril (□, Group III, $N = 6$), TA-3090 (▲, Group IV, $N = 5$), or both (■, Group V, $N = 4$). Compared to control dogs, values for BG were comparably elevated ($P < 0.05$) in all groups of diabetic dogs. Values for Groups I and II are means ± SEM. For Groups III to V only means are depicted. *$P < 0.05$ verses Groups II to V.

antihypertensive agents averaged 0.7 mg lisinopril/kg/day and 15.7 mg TA-3090/kg/day.

Endogenous creatinine clearance was not significantly different among groups of diabetic dogs at any time point in the study and the mean value in all groups of diabetic dogs was significantly ($P < 0.05$) greater than that in Group I at 8 and 12 months (Fig. 3). Compared to Group I, the mean final serum creatinine concentration was significantly reduced in all four groups of diabetic dogs (Table 1). These final values were not different from the mean values for serum creatinine (mg/dl) obtained in the first month of the study: 1.2 ± 0.3 (Group I), 0.8 ± 0.1 (Group II), 1.0 ± 0.7 mg/dl (Group III), 0.9 ± 0.1 (Group IV), and 0.8 ± 0.1 mg/dl (Group V). There were no significant differences among groups in blood urea nitrogen concentration, hematocrit, or plasma total protein concentration (Table 1).

Renal hemodynamic studies (Groups I to IV)

During renal clearance and micropuncture studies conducted at spontaneous RAP in Groups I to IV (Table 2), the plasma glucose concentration was similarly elevated in the three groups of diabetic dogs. The RBF and GFR were also significantly elevated in Groups II, III, and IV. Dogs in Groups III and IV exhibited intermediate values for GFR and RBF which were not significantly different from untreated diabetics. Compared to Group I, dogs of Group II exhibited a significant elevation in FF. The chronic administration of lisinopril to diabetic dogs (Group III) resulted in a value for FF that was significantly ($P < 0.05$) lower than that observed in untreated diabetic dogs (Group II) but not different from the mean value for dogs of Group I. All three groups of diabetic dogs exhibited a comparable elevation in urinary flow rate.

Micropuncture studies (Table 3) indicated that SNGFR of outer cortical nephrons was elevated ($P < 0.05$) in untreated diabetic dogs, with a mean increase of 63%. A similar degree of hyperfiltration was observed in Groups III and IV. Compared to Group I, Group II exhibited a lower mean free-flow proximal tubule pressure and an elevation in SFP, resulting in a significant increment in both P_{GC} and ΔP. Group III dogs exhibited mean values for SFP, P_{GC}, and ΔP that were reduced from those observed in Group II and indistinguishable from values

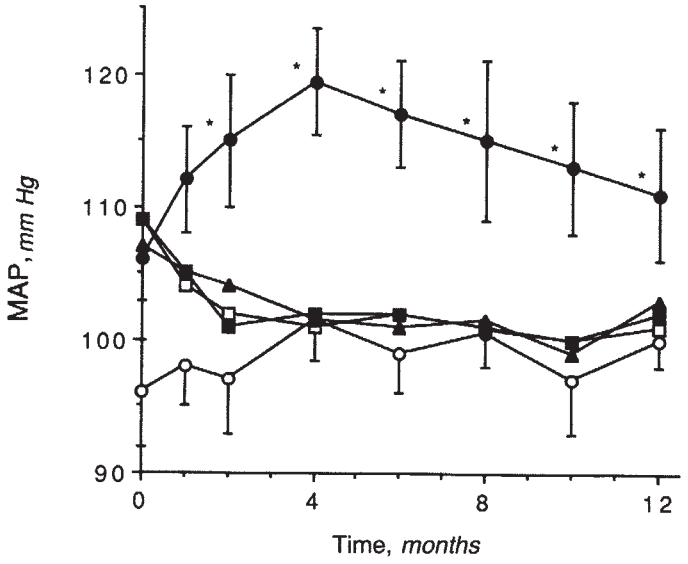

Fig. 2. *Serial values for mean arterial pressure (MAP) measurements in awake dogs.* Serial values for MAP in control dogs (○, Group I, N = 6), untreated diabetic dogs (●, Group II, N = 8), or diabetic dogs treated with lisinopril (□, Group III, N = 6), TA-3090 (▲, Group IV, N = 5), or both (■, Group V, N = 4). Compared to control dogs, values for MAP were mildly elevated ($P < 0.05$) in untreated diabetic dogs and comparably lowered in Groups III to V. Values for Groups I and II are means ± SEM. For groups III to V only means are depicted. *$P < 0.05$ verses Group I.

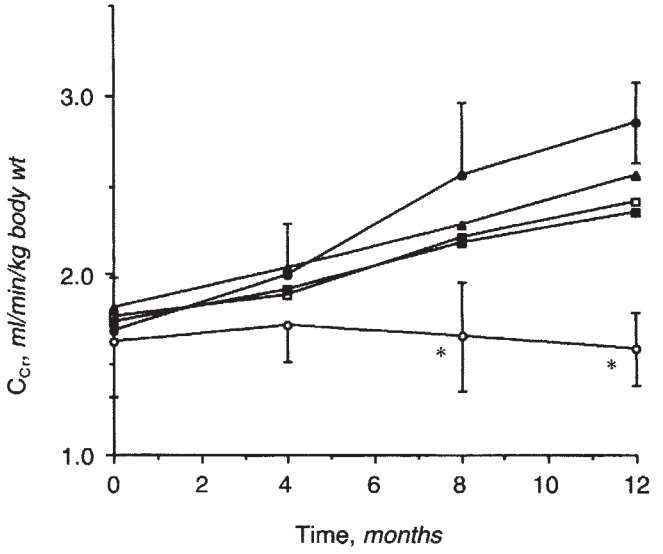

Fig. 3. *Serial values for endogenous creatinine clearance (C_{Cr}) measurements in dogs.* Serial values for C_{Cr} in control dogs (○, Group I, N = 6), untreated diabetic dogs (●, Group II, N = 8), or diabetic dogs treated with lisinopril (□, Group III, N = 6), TA-3090 (▲, Group IV, N = 5), or both (■, Group V, N = 4). Compared to control dogs, values for C_{Cr} were elevated ($P < 0.05$) in all 4 groups of diabetic dogs. Values for Groups I and II are means ± SEM. For groups III to V only means are depicted. *$P < 0.05$ versus Groups II to V.

for Group I. Compared to Group II, dogs treated with TA-3090 (Group IV) exhibited significantly increased free-flow PTP, but there was no significant difference between Groups II and IV in SFP, P_{GC}, or ΔP. There were no significant differences in PCP or πa among groups.

Total renal vascular resistance (R_T) was 0.39 ± 0.05 nl · min^{-1} · mm Hg^{-1} in Group I and was reduced ($P < 0.05$) to a mean value of 0.29 ± 0.02 nl · min^{-1} · mm Hg^{-1} in Group II, 0.24 ± 0.01 nl · min^{-1} · mm Hg^{-1} in Group III, and 0.26 ± 0.04 nl · min^{-1} · mm Hg^{-1} in Group IV. Mean values for RA (Table 3) in Groups II to IV were less than ($P < 0.05$) the corresponding value in Group I. The differences in mean values for R_T and R_A between treated and untreated diabetic dogs did not reach statistical significance. Chronic administration of the ACEI, lisinopril, led to a mean value for R_E in Group III that represented a 30% reduction ($P < 0.05$) from that observed in Group II. Although there was a trend for K_f to increase in both treated groups, the K_f did not differ significantly among groups.

Autoregulation studies (Groups I to IV)

Following renal micropuncture studies, renal autoregulatory capability was assessed. Initial measurements of GFR and RBF were made at spontaneous values for MAP (Table 1). In dogs in which spontaneous MAP exceeded 100 mm Hg, the RAP was reduced to approximately 100 mm Hg and clearance measurements were repeated. These studies were conducted at similar mean values for MAP of 99.5 ± 2.4 mm Hg in Group I (N = 5), 104.6 ± 2.0 mm Hg in Group II (N = 7), 100.1 ± 2.2 mm Hg in Group III (N = 5), and 103.8 ± 1.5 mm Hg in Group IV (N = 4). At the reduced RAP, mean values for RBF and GFR were: 11.7 ± 0.7 and 1.84 ± 0.06 ml/min/kg body wt in Group I, 15.9 ± 1.6 and 3.06 ± 0.37 ml/min/kg body wt in Group II, 16.9 ± 2.5 and 2.70 ± 0.38 ml/min/kg body wt in Group III, and 14.0 ± 2.0 and 2.57 ± 0.28 ml/min/kg body wt in Group IV. Autoregulation of GFR and RBF was assessed by determination of the autoregulatory index, which represents the ratio of % change in measured parameter (RBF or GFR) divided by % change in MAP. An autoregulation index of 0 indicated perfect autoregulation, and an index of 1.0 indicated a lack of autoregulation and a passive relationship between MAP and RBF or GFR. These results indicated a reduced capability to autoregulate RBF and

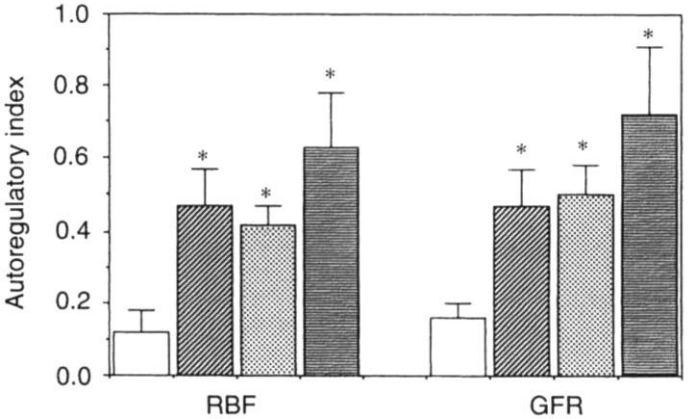

Fig. 4. *Renal autoregulatory indices.* Values for renal autoregulatory indices for renal blood flow (RBF) and glomerular filtration rate (GFR) in control dogs (□, Group I, N = 6), untreated diabetic dogs (▨, Group II, N = 8), or diabetic dogs treated with lisinopril (▩, Group III, N = 6) and TA-3090 (▤, Group IV, N = 5). Compared to Group I, renal autoregulatory efficiency for both RBF and GFR was reduced in Groups II to IV. Values are means ± SEM. *$P < 0.05$ versus Group I.

Table 3. Results of micropuncture studies 1 year after the induction of diabetes mellitus in dogs

Group	N	SNGFR	SFP	PTP	PCP	π_a	P_{GC}
		nl/min	*mm Hg*				
Group I	6	52.1 ± 3.6	37.5 ± 2.0	18.0 ± 1.4	8.1 ± 0.7	16.0 ± 0.6	54.3 ± 2.0
Group II	8	85.1 ± 8.1[a]	45.4 ± 1.5[a]	13.6 ± 1.1[a]	7.0 ± 0.7	17.9 ± 0.5	62.8 ± 1.7[a]
Group III	6	80.7 ± 5.4[a]	37.1 ± 1.7[b]	13.7 ± 1.5[a]	8.5 ± 0.6	17.8 ± 0.7	55.1 ± 1.9[b]
Group IV	5	82.3 ± 3.7[a]	45.1 ± 2.1[a,c]	18.2 ± 1.6[b,c]	8.9 ± 1.2	18.0 ± 0.6	63.4 ± 1.1[a,c]

Values are means ± SEM. Abbreviations are: SNGFR, single nephron glomerular filtration rate; SFP, proximal tubule stop-flow pressure; PTP, proximal tubule free-flow pressure; πa, afferent colloid osmotic pressure; P_{GC}, glomerular capillary hydrostatic pressure; ΔP, glomerular transcapillary hydrostatic pressure gradient; R_A, afferent arteriolar resistance; R_E, efferent arteriolar resistance; K_f, glomerular ultrafiltration coefficient.

[a] $P < 0.05$ vs. Group I
[b] $P < 0.05$ vs. Group II
[c] $P < 0.05$ vs. Group III

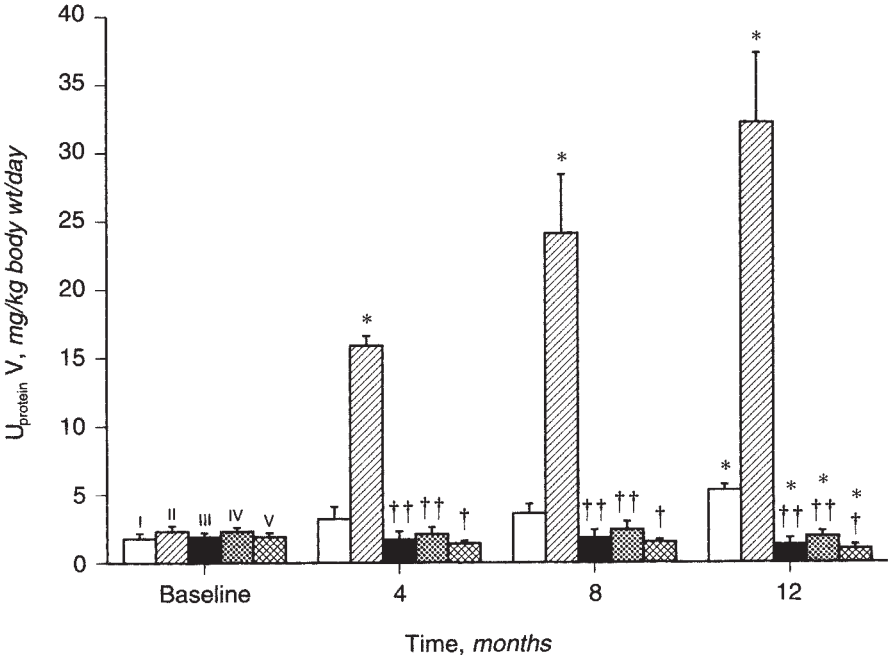

Fig. 5. *Serial values for daily urinary protein excretion ($U_{prot}V$) in awake dogs.* Serial values for $U_{prot}V$ in control dogs (□, Group I, $N = 6$), untreated diabetic dogs (▨, Group II, $N = 8$), or diabetic dogs treated with lisinopril (■, Group III, $N = 6$), TA-3090 (▩, Group IV, $N = 5$), or both (▩, Group V, $N = 4$). Compared to control dogs, values for $U_{prot}V$ were elevated ($P < 0.05$) in untreated diabetic dogs. The rise in proteinuria was attenuated in Groups III to V. Moreover, combination therapy resulted in the greatest attenuation of proteinuria in Group V. Values are means ± SEM. *$P < 0.05$ versus baseline, †$P < 0.05$ versus all other groups, ††$P < 0.05$ versus Group II.

GFR in Group II (Fig. 4). While chronic converting enzyme inhibition (Group III) had no apparent additional effect on renal autoregulation, dogs in Group IV exhibited a nonsignificant trend for a further decrement in renal autoregulatory capability.

Glomerular volume determinations (Groups I to V)

The mean glomerular volume was 3.52 ± 0.13 × $10^6\mu^3$ in Group I. Glomerular enlargement was observed in all four groups of diabetic dogs ($P < 0.05$). However, compared to the mean glomerular volume in Group II of 6.14 ± 0.30 × $10^6\mu^3$, treatment of diabetic dogs with lisinopril, TA-3090, or both significantly ($P < 0.05$) limited the extent of glomerular hypertrophy and corresponding values for mean glomerular volume were 4.57 ± 0.36 × $10^6\mu^3$ in Group III, 4.74 ± 0.27 × $10^6\mu^3$ in Group IV, and 4.55 ± 0.86 × $10^6\mu^3$ in Group V.

Proteinuria (Groups I to V)

Significant increments in proteinuria were present in Group II at all time points (Fig. 5). Treatment with lisinopril and TA-3090 attenuated the increase of proteinuria to a value that was not significantly different from the mean value in Group I. Interestingly, dogs treated with combination therapy (Group V) exhibited a further decrement ($P < 0.05$) in urinary protein excretion. Furthermore, the mean value in this group was significantly less than that observed in uninephrectomized control dogs (Group I).

Discussion

Diabetes mellitus induced in dogs by alloxan administration was associated with moderate hyperglycemia, systemic hypertension, and significant changes in renal function. Compared to controls, untreated diabetic dogs exhibited whole organ hyperperfusion and hyperfiltration. Micropuncture measurements in outer cortical nephrons demonstrated single nephron hyperfiltration in untreated diabetic dogs which was attributable to a fall in preglomerular vascular resistance and a resultant increase in P_{GC} and ΔP. These hemodynamic changes were accompanied by glomerular hypertrophy and substantial proteinuria. Treatment with lisinopril, TA-3090, or both led to modest, comparable reductions in MAP among the treated

Table 3. Continued

ΔP	R_A	R_E	K_f
mm Hg	*mm Hg · min · nl^{-1}*		*ml · min^{-1} · mm Hg^{-1}*
36.3 ± 2.0	0.205 ± 0.027	0.186 ± 0.023	3.53 ± 0.40
49.3 ± 2.0[a]	0.142 ± 0.014[a]	0.159 ± 0.012	3.61 ± 0.32
41.4 ± 1.4[b]	0.126 ± 0.011[a]	0.110 ± 0.007[a,b]	4.53 ± 0.48
45.2 ± 1.2[a]	0.120 ± 0.018[a]	0.153 ± 0.021	4.12 ± 0.23

diabetic dogs. All treatment regimens were associated with significant reductions in the extent of glomerular hypertrophy and proteinuria, without significantly lowering RBF, GFR, or SNGFR.

Therapy with either lisinopril or TA-3090 alone, however, had contrasting effects on glomerular hydraulic pressures. The chronic administration of lisinopril to diabetic dogs produced a decrement in postglomerular resistance which led to a reduction in both P_{GC} and ΔP to values similar to those observed in nondiabetic control dogs. Similarly, previous studies in rats have consistently demonstrated that ACEI administration lowers P_{GC} in diabetic rats and in rats with reduced renal mass [8, 9, 11]. A recent study indicated no effect of the dihydropyridine-type CA nifedipine on P_{GC} in diabetic rats [11]. While treatment of diabetic dogs with TA-3090 similarly had no apparent effect on P_{GC} in diabetic dogs, this CA did produce a significant increase in free-flow PTP, resulting in a value for ΔP that was reduced, albeit not statistically different, from the mean value for ΔP observed in untreated diabetic dogs. A similar differential effect of CA on P_{GC} and ΔP has been associated with renoprotection in rats with reduced renal mass [33]. In contrast, treatment of diabetic rats with the CA nifedipine produced a similar increment in proximal tubular free-flow pressure without reducing P_{GC}, ΔP, or the extent of glomerular injury [11]. The mechanism responsible for variations in PTP in the dogs of this study is unknown.

The different effects of lisinopril and TA-3090 on glomerular capillary pressure were attributed to effects of these agents on segmental renal resistances. Diabetic dogs treated with lisinopril, but not TA-3090, exhibited a substantial decrement in postglomerular resistance. Although both antihypertensive agents tended to produce a decrement in afferent arteriolar tone, neither of these trends was statistically significant. Previous studies in normal dogs provide evidence for a differential effect of acutely administered CA and ACEI on segmental renal vascular resistances, with CA preferentially lowering preglomerular vascular resistance [34] and ACEI producing afferent and efferent vasodilation [35, 36]. Apparently contrasting results were obtained in one study which indicated that diltiazem and verapamil lowered P_{GC} in partially nephrectomized rats [37]. However, the fall in P_{GC} in CA treated rats of that study was attributable to a marked decline (≥45 mm Hg) in MAP rather than to a preferential lowering of R_E. In contrast, treatment of diabetic dogs with CA in the current study produced a comparatively mild decrement in MAP coupled with the absence of a significant effect on R_E and consequently the CA did not alter P_{GC}.

We demonstrated an impairment of renal autoregulatory control of RBF and GFR above 100 mm Hg in diabetic dogs. Although limited, these studies are consistent with findings in normal dogs during intrarenal infusion of glucose [38]. Studies in diabetic rats have provided conflicting evidence of either impaired [39] or enhanced [40] RBF autoregulation. The alterations of renal autoregulation in diabetic animals may, in part, be due to a failure of myogenic vasoconstriction [41] or to an alteration in the macula densa feedback signal [38].

Many studies of single nephron hemodynamics in diabetic animals have been conducted in Munich-Wistar rats, primarily because of the availability of superficial glomeruli for direct micropuncture measurement of P_{GC}. In the dog, superficial glomeruli are rarely available, so proximal tubule SFP is generally used to estimate P_{GC} [29, 30, 42, 43]. Canine P_{GC} estimated from SFP measurements are, in general, slightly higher than the direct measurements of P_{GC} in this species [43–45]. Similarly, SNGFR determined by total proximal collection results in an overestimation of true SNGFR, as assessed by distal collections [46]. Since the current study employed the same technique to measure P_{GC} and SNGFR in all dogs studied by micropuncture, it is unlikely that methodological considerations could account for the observed differences.

Several factors may have contributed to observed reductions in proteinuria in the treatment groups. Dogs chronically treated with lisinopril exhibited a reduction in MAP, P_{GC}, ΔP, and glomerular volume associated with a decline in proteinuria. The attenuation of proteinuria and preservation of glomerular structure in diabetic rats has been causally associated with the lowering of P_{GC} and/or ΔP observed in rats chronically treated with ACEI [8–11]. However, monotherapy of diabetic dogs with CA produced a decrement in proteinuria that was similar to that observed in diabetic dogs treated with lisinopril and associated with a significant reduction in glomerular volume and MAP but with no effect on P_{GC}. These results do not, however, determine whether the observed proteinuria was associated with structural glomerular lesions or alterations in glomerular basement membrane charge or size selectivity.

While glomerular hypertension and a loss of renal autoregulatory capability were observed in diabetic dogs, this study did not investigate the long-term consequences of the observed changes in renal hemodynamics. Within the time course of the current study, diabetic dogs demonstrated a temporal pattern of increasing hyperfiltration. The lack of progressive decrements of GFR is not surprising, since previous studies in nondiabetic dogs indicate that a similar degree of glomerular hypertension does not produce rapid decrements in GFR [30, 47–53], suggesting that the hypertensive canine glomerulus may be less susceptible than that of rodents to the development of glomerulosclerosis. However, our studies demonstrated increased proteinuria in diabetic dogs and further showed that the extent of the proteinuria was reduced by antihypertensive therapy.

In summary, diabetic dogs exhibited systemic hypertension, proteinuria, glomerular hyperfiltration, glomerular hypertension, glomerular hypertrophy, and a loss of renal autoregulatory capability. Dogs treated with lisinopril had normalization of arterial and glomerular capillary pressures and glomerular volume. Treatment with TA-3090 resulted in equivalent control of arterial pressure and glomerular volume but had no apparent effect on P_{GC}. Treatment of diabetic dogs with the CA did attenuate the rise in proteinuria to a similar extent as the ACEI. Lastly, combining the two antihypertensive agents resulted in the greatest antiproteinuric effect. In these diabetic dogs, there

was an association between the extent of glomerular enlargement and proteinuria. However, these data demonstrate a dissociation between extent of proteinuria and control of P_{GC}. Our results are consistent with the concept that ACEI and CA have different effects on renal hemodynamics and, further, that the attenuation of proteinuria in diabetics treated with antihypertensive agents is not solely dependent upon a reduction in glomerular capillary pressure.

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