Unselective Inhibition of Endothelin Receptors Reduces Renal Dysfunction in Experimental Diabetes

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Chronic nephropathies are associated with enhanced renal synthesis of endothelin (ET)-1. A recent study demonstrated that an ET_A receptor antagonist given to diabetic rats at the moment of disease induction prevented the development of renal injury. Here we investigated whether an unselective ET_A/ET_B receptor antagonist, PD 142,893, was renoprotective when given to streptozotocin diabetic rats when animals were already proteinuric. The effect of PD 142,893 was compared with that of an ACE inhibitor, lisinopril, known to retard progressive renal disease in experimental and human diabetes. PD 142,893 normalized systemic blood pressure, reduced urinary protein and albumin excretion, and ameliorated renal blood flow in diabetic rats, but it did not affect such parameters in control rats. Lisinopril had a renoprotective effect comparable to PD 142,893, although lisinopril controlled systemic blood pressure better. Northern blot analysis of ET-1 mRNA revealed upregulation of ET-1 gene in the diabetic kidney. Similar results were obtained by in situ hybridization in glomeruli and tubuli of diabetic rats. Both treatments remarkably attenuated exaggerated renal ET-1 gene expression. These data suggest that ET-1 is a contributory mediator of kidney damage in diabetes and indicate that ET receptor antagonists may represent a new therapeutic mean for treatment of progressive diabetic nephropathy. *Diabetes* 47:450–456, 1998

A number of recent studies documented that renal synthesis of endothelin (ET)-1 is increased in chronic nephropathies either in experimental animals or in humans (1–4) and that mice and rats transgenic for ET-1 or ET-2 are phenotypically characterized by renal lesions (5,6). The vasoactive and inflammatory potential of ET-1 inspired studies to explore its potential role as a mediator of renal damage. Thus, chronic administration of ET receptor antagonists selectively binding either to ET_A or to both ET_A and ET_B receptors protects animals from developing renal injury (7,8). In a model of immune nephritis induced by repeated injections of ovoalbumin, selective blockade of ET_A and ET_B receptors effectively reduced proteinuria and protected animals from renal function impairment (9). Consistent with findings in nondiabetic models of renal disease progression, Nakamura et al. (10) have recently found that a selective antagonist for ET_A receptor given to diabetic rats at the moment of the induction of the disease effectively prevented the development of renal disease and attenuated the increase in serum creatinine. However, no data are available so far on whether an ET receptor antagonist is capable of controlling renal disease progression if given therapeutically to animals that already have clinical signs of diabetic nephropathy. Here we studied the potential renoprotective properties of an unselective ET receptor antagonist, PD 142,893, chronically administered to streptozotocin diabetic animals starting from 6 months after diabetes induction, when animals had developed overt proteinuria. The effect of antagonizing ET receptors was formally compared with that of ACE inhibitors, the best means available so far to control protein excretion and renal disease progression in experimental and human diabetes (11,12).

RESEARCH DESIGN AND METHODS

Pilot study. A preliminary study was performed to check whether PD 142,893 [AC-(D)-DIP-(L)-LEU-(L)-ASP-(L)-ILE-(L)-ILE-(L)-TRP] (13), a peptidic ET_A/ET_B receptor antagonist, at a dose of 3 $\mu mol \cdot kg^{-1} \cdot day^{-1}$, was effective in preventing ET-1–induced increase in systolic blood pressure. Three groups of male Sprague-Dawley rats (Charles River, Calco, Italy), weighing 225–250 g, were treated intraperitoneally for three consecutive days: group 1 ($n = 6$) received 5% glucose (vehicle) and groups 2 ($n = 6$) and 3 ($n = 6$) received PD 142,893 (3 $\mu mol \cdot kg^{-1} \cdot day^{-1}$). The experiments described below were performed 3 (group 2) or 24 h (group 3) after the last administration of the active drug. Animals were anesthetized with tiopenthal sodium (60 mg/kg i.p.). A polyethylene (PE) 240 cannula was inserted into the trachea to facilitate breathing; a PE 50 cannula was placed in the left femoral vein for bolus injection of ET-1; and a PE 50 cannula was advanced into the left femoral artery to measure arterial blood pressure with a Statham pressure transducer connected to a carrier amplifier (Battaglia Rangoni, Bologna, Italy). Rats were allowed to equilibrate for 30-min intervals. Increasing doses of ET (70, 140, 300 pmol) were given as bolus injections at 30-min intervals. Systolic blood pressure was monitored thereafter. Percent changes in mean arterial pressure induced by ET-1 were calculated from data obtained 10–20 min after injection of the peptide versus the initial control period.

Experimental design. Thirty-two adult male Sprague-Dawley rats, with initial body weights of 250–275 g, were used. Animal care and treatment were conducted in compliance with institutional guidelines and national and international laws and policies (EEC Council Directive 86/609, OJL 358, Dec 1987; *NIH Guide for the Care and Use of Laboratory Animals*, NIH Publication No. 85-83, 1985). All animals were allowed free access to food (standard rat laboratory diet containing 20% protein by weight) and tap water.

Twenty-four rats were made diabetic by a single intravenous tail injection of streptozotocin (Sigma, St. Louis, MO), 60 mg/kg body wt, under light ether anesthesia. The remaining eight rats served as controls. The induction of diabetes was

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ET, endothelin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFR, glomerular filtration rate; PAH, *p*-aminohippurate; PE, polyethylene; RPF, renal plasma flow.

confirmed 2 days later by measurement of tail blood glucose level using a reflectance meter (Miles Laboratories, Elkhart, IN). Diabetic rats received daily evening injections of ultralente insulin (Ultralente MC Novo, Industry, Copenhagen, Denmark) in doses individually adjusted to maintain blood glucose levels between 200 and 400 mg/dl. Blood glucose levels were monitored at least once a week in all diabetic rats and occasionally in nondiabetic rats for comparison purposes. Animals were followed for 6 months, until urinary protein excretion reached the value of 40–50 mg/day. Until this period, diabetic rats received no therapy other than insulin. Then animals were grouped as described below and followed for 4 weeks: group 4 (n = 8) received no treatment; group 5 (n = 8) received PD 142,893 (3 µmol · kg^{-1} · day^{-1}) twice a day intraperitoneally; and group 6 (n = 8) received the ACE inhibitor lisinopril (40 mg/l) in the drinking water. Normal rats (group 7) with no treatment were also followed for the same time period.

In all groups, blood pressure and urinary protein excretion were measured before the induction of diabetes (time 0), at days 120 and 195 after streptozotocin injection, and every 15 days (days 210 and 225) after the beginning of treatment. At the end of the study period, whole-kidney function was measured through glomerular filtration rate (GFR) and renal plasma flow (RPF). After the rats were killed, their kidneys were removed and processed for in situ hybridization for ET-1 and histological analysis as well as for RNA extraction and Northern blot analysis of ET-1.

The effect of PD 142,893 was also evaluated in additional control rats (n = 6) given the ET receptor antagonist (3 µmol/kg) twice a day intraperitoneally for 6 days. Blood pressure, urinary protein excretion, and renal function were assessed before and after treatment in this latter group.

RNA isolation and Northern blot analysis. Total RNA was isolated from rat kidney by the guanidium isothiocyanate/cesium chloride procedure as previously described (1). For mRNA preparation, total RNA of kidneys of each experimental group was pooled together. Poly(A)+ RNA was selected by oligo (dT)-cellulose column chromatography (mRNA separator; Clontech, Palo Alto, CA). Ten micrograms of mRNA were then fractionated on 1.2% formaldehyde agarose gel and blotted onto nylon filters (Zeta-probe; Biorad, Richmond, CA). Rat ET-1 cDNA was labeled with α^{32}P-dCTP by random primed method (1). Hybridization was performed overnight in 50% formamide, 0.12 mol/l Na_2HPO_4, pH 7.2, 0.25 mol/l NaCl, and 7% SDS at 43°C. Filters were washed 3 times for 15 min with 2× SSC and 0.1% SDS at room temperature, for 15 min with 0.5× SSC and 0.1% SDS at room temperature, and for 15 min with 0.1× SSC and 0.1× SDS at 65°C. Membranes were subsequently probed with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) taken as the internal standard of equal loading of the samples on the membrane. ET-1 mRNA optical density was normalized to that of the constitutive GAPDH gene expression. Values were expressed as optical density units relative to the specific mRNA levels in control rats.

Non-isotopic in situ hybridization. Rat ET-1 antisense and sense probes were prepared and labeled by in vitro transcription of a 319-bp rat ET-1 subcloned into a pGEM7zf (+) vector (kindly provided by Dr. E. Schiffrin, Quebec, Canada) using a digoxigenin RNA labeling kit (Boehringer Mannhein, Mannhein, Germany). Before use, digoxigenin-labeled riboprobes were checked for size, integrity, and labeling efficiency.

Renal tissue fixed in 10% neutral formalin and embedded in paraffin was cut at 4 µm and deparaffinized. After incubation with 5 mmol/l Levamisole and permeabilization with HCl 0.2 N and proteinase K (40 µg/ml, Sigma), sections (n = 5 for each animal) were hybridized overnight with a mixture consisting of 0.5 ng/µl of digoxigenin-labeled RNA probe, 2× SSC, 10% dextran sulfate, 1× Denhardt's solution, and 0.1 mol/l sodium phosphate in a moist chamber at 42°C. Slides were then washed in 0.2× SSC, treated with a blocking solution (50 mg/ml skimmed dried milk, 150 mmol/l NaCl in 100 mmol/l Tris HCl, pH 7.8), and incubated with anti-digoxigenin antibody conjugated with alkaline phosphatase (Boehringer Mannheim). Sections were finally colored with freshly prepared nitroblue tetrazolium and X-phosphate-5-bromo-4-chloro-3-indolyl phosphate. Negative controls were performed using the sense probe.

Other analyses. Systolic blood pressure was evaluated by tail cuff plethysmography (14). Protein concentration in the urine was measured by the Coomassie blue G dye-binding method (15). Urinary albumin concentration was determined by enzyme-linked immunosorbent assay (ELISA) as described previously (16). Fifty microliters of urine sample (1:6,000–1:10,000 dilution) were added to a rabbit anti-rat albumin antibody–coated plate (Cappel-DBH, Milan, Italy). After 60 min incubation, a specific sheep anti-rat albumin antibody conjugated with peroxidase was added (Cappel-DBH, dilution 1:2,500). Substrate reaction was started with the addition of 0.137% o-phenylenediamine and stopped with 1 mol/l H_2SO_4. Absorbance was determined at 490 nm in a microtiter plate reader. Renal function was assessed in anesthetized rats by inulin and p-aminohippurate (PAH) clearances as previously described (17).

Inulin concentration in plasma and urine samples was measured by the thiourea-resorcinol method (18). PAH concentration in plasma and urine was determined by the method of Smith et al. (19). GFR and RPF, measured as inulin and PAH clearance, respectively, were calculated using the standard formula. Histological evaluation was performed in Dubosq-Brazil–fixed, paraffin-embedded kidney fragments stained with Masson's trichrome, hematoxylin and eosin, and periodic-acid Schiff reagent (PAS stain).

Statistical analysis. Results are expressed as means ± SD. Data were analyzed by repeated measures analysis of variance using the GLM procedure of the SAS program (20). Multiple comparisons between groups were carried out using the Tukey's studentized range test (21). Values of proteinuria and urinary albumin excretion were log transformed before statistical analysis. Statistical difference for GFR and RPF was assessed by Tukey-Cicchetti test. Statistical significance level was defined as $P < 0.05$.

RESULTS

Pilot study. Of the two groups of animals treated with PD 142,893 for 3 days and then challenged with ET-1 (70, 140, and 300 pmol) 3 or 24 h after the last administration of the drug, the former group had a higher blood pressure reduction than did the latter one, suggesting that the compound has a quite short duration of action (Fig. 1). This prompted us to administer it twice a day in all subsequent experiments.

Chronic administration of PD 142,893 to control rats. To evaluate the possible effect of PD 142,893 on control rats, animals were given the compound for 6 days. Blood pressure and urinary protein excretion rate were not modified by the treatment (blood pressure before, 139 ± 8; after, 139 ± 6 mmHg; urinary protein before, 21 ± 6; after, 20 ± 3 mg/day). Similarly, PD 142,893 did not affect GFR and RPF (GFR 2.1 ± 0.2 ml/min; RPF 6.8 ± 1.0 ml/min).

Chronic administration of PD 142,893 or lisinopril to diabetic rats. Food and water intake were significantly higher in diabetic than in control rats throughout the study and were not modified by PD 142,893 and lisinopril administration. Stable moderate hyperglycemia was maintained throughout the duration of the experiment. The three groups of diabetic rats had comparable levels of blood glucose during all the observation periods. Values of blood glucose at 120 days averaged 383 ± 34 mg/dl in untreated diabetic animals;

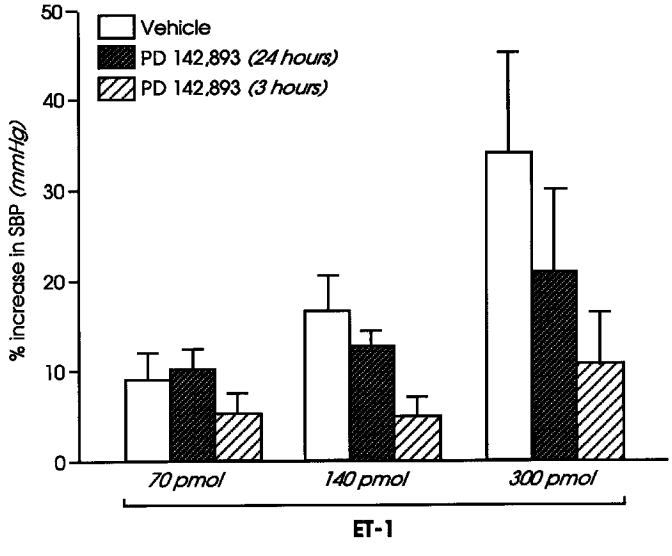

FIG. 1. Prevention of blood pressure increase induced by 70, 140, and 300 pmol ET-1 with PD 142,893. Animals (n = 6 for each group) were treated for 3 days with vehicle or 3 µmol · kg^{-1} · day^{-1} PD 142,893, and experiments were performed 3 or 24 h after the last administration of PD 142,893. Data are means ± SD.

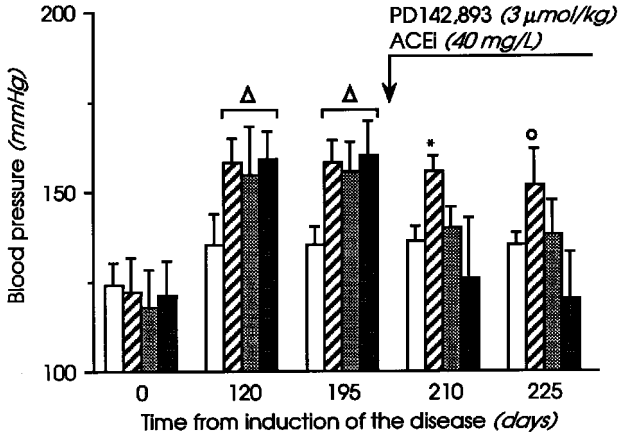

FIG. 2. Periodic assessments of systolic blood pressure in controls (□) and in diabetic rats given vehicle (▨), PD 142,893 (▩), or lisinopril (■). Data are means ± SD. Determinations were performed in 8 animals for each group at all time points except at 225 days, when only 7 animals in each diabetic group survived. △, $P < 0.01$ vs. controls; *, $P < 0.05$ vs. controls, diabetic + PD 142,893, and diabetic + lisinopril; ○, $P < 0.05$ vs. controls, diabetic + lisinopril.

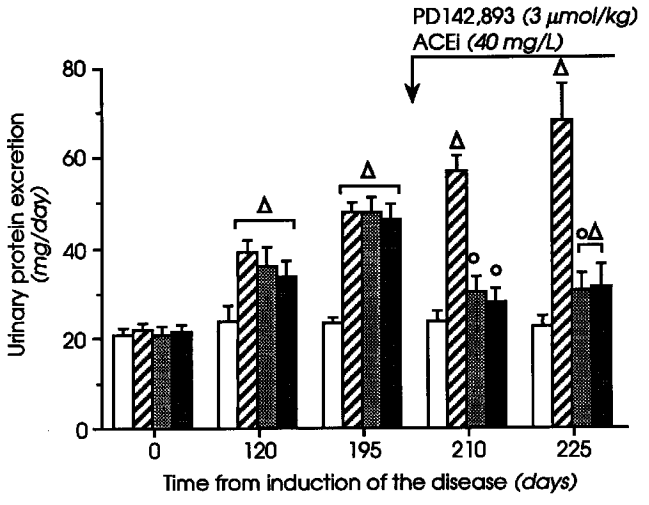

FIG. 3. Time course of urinary protein excretion in controls (□), diabetic rats (▨), and diabetic rats given PD 142,893 (▩) or lisinopril (■). Data are means ± SD. Determinations were performed in 8 animals for each group at all time points except at 225 days, when only 7 animals in each diabetic group survived. △, $P < 0.05$ vs. controls; ○, $P < 0.05$ vs. diabetics.

344 ± 45 and 395 ± 10 mg/dl in PD 142,893–treated and lisinopril-treated animals; and 91 ± 12 mg/dl in control rats. At the end of the experimental period, blood glucose levels were not affected either by PD 142,893 (375 ± 40 mg/dl) or by lisinopril (386 ± 27 mg/dl) and were comparable to untreated diabetic animals (377 ± 40 mg/dl). Body weight was significantly lower in diabetic than in control animals at 225 days (540.6 ± 25.2 vs. 655.5 ± 43.6 g, $P < 0.01$). Treatment of diabetic rats with PD 142,893 or lisinopril did not ameliorate weight gain (PD 142,893: 576.4 ± 63.0 g; lisinopril: 569.6 ± 60.0 g, $P < 0.05$ vs. controls). Kidney weight was higher in diabetic than in control rats (2.51 ± 0.31 vs. 2.01 ± 0.16 g, $P < 0.01$) and remained higher than in controls in diabetic rats given ET receptor antagonist (2.42 ± 0.23 g, $P < 0.05$) and ACE inhibitor (2.41 ± 0.16 g, $P < 0.05$). One rat in each group of diabetic animals died during the course of the experiment.

Blood pressure. At 120 days, all three groups of diabetic rats developed hypertension (diabetic: 158 ± 7; diabetic + PD 142,893: 155 ± 13; diabetic + lisinopril: 159 ± 8 vs. control: 135 ± 9 mmHg, $P < 0.01$) (Fig. 1). The increase in blood pressure persisted at 195 days (diabetic: 158 ± 7; diabetic + PD 142,893: 156 ± 8; diabetic + lisinopril: 160 ± 10 vs. control: 135 ± 5 mmHg, $P < 0.01$) (Fig. 2). Fifteen days after the beginning of treatment, either PD 142,893 or lisinopril significantly lowered systolic blood pressure as compared with untreated diabetic rats (diabetic + PD 142,893: 140 ± 6; diabetic + lisinopril: 126 ± 17 vs. diabetic: 156 ± 4 mmHg, $P < 0.05$) to levels that were not significantly different from those of controls (136 ± 4 mmHg). Lisinopril controlled blood pressure better than did PD 142,893 ($P < 0.05$). At the end of the study, blood pressure in PD 142,893–treated animals was only numerically but no longer significantly reduced relative to that in untreated diabetic animals (139 ± 10 vs. 152 ± 10 mmHg). By contrast, lisinopril better controlled blood pressure, further reducing it to values that were even lower than control values (120 ± 13 vs. 136 ± 3 mmHg, $P < 0.05$) (Fig. 2).

Urinary protein excretion. The time course of urinary protein excretion is depicted in Fig. 3. Baseline proteinuria values were similar in all animals. At 120 days, diabetic animals developed significant proteinuria compared with controls ($P < 0.05$). Urinary protein excretion further increased over control values at 195 days ($P < 0.05$). Fifteen days after treatment, both treatments significantly prevented the further increase in proteinuria that was occurring in diabetic untreated rats (PD 142,893: 30 ± 4; PD 142,893 + lisinopril: 28 ± 3 vs. diabetic: 57 ± 3 mg/day, $P < 0.05$). Values of urinary proteins in diabetics were significantly higher than in controls (24 ± 3 mg/day, $P < 0.05$). At the end of the study, PD 142,893– and lisinopril-treated diabetic animals were still significantly protected from the development of proteinuria relative to untreated diabetic rats (diabetic + PD 142,893: 31 ± 4; diabetic + lisinopril: 31 ± 5 vs. diabetic: 68 ± 8 mg/day, $P < 0.05$), although values of both groups were still significantly higher than those of control rats (23 ± 2 mg/day, $P < 0.05$).

The time course of urinary albumin excretion is depicted in Fig. 4. Basal values were similar in all groups. Absolute values of urinary albumin excretion were higher in diabetic animals than in controls at 120 and 195 days, but the trend was statistically significant ($P < 0.05$) only at the latter time. At the end of the study, urinary albumin excretion was significantly higher in diabetic versus control rats (14.5 ± 4.7 vs. 1.1 ± 0.32 mg/24 h, $P < 0.05$). Both treatments reduced urinary albumin excretion, although only lisinopril reduced it to a statistically significant extent (lisinopril: 3.9 ± 2.2 mg/24 h, $P < 0.05$; PD 142,893: 9.9 ± 2.3 mg/24 h).

Renal ET-1 mRNA expression. ET-1 gene expression in kidneys from diabetic rats and the effect of blocking angiotensin II formation and ET-1 biological activity are depicted in Fig. 5. Densitometric analysis of the autoradiographic signals showed that ET-1 transcript levels in diabetic rats were twofold higher than those in control rats. Both the ACE inhibitor and the ET receptor antagonist attenuated the increase in mRNA levels for ET-1 (0.2 and 0.3, respectively).

Localization of ET-1 mRNA in the kidney. In situ hybridization was performed to evaluate ET-1 mRNA distri-

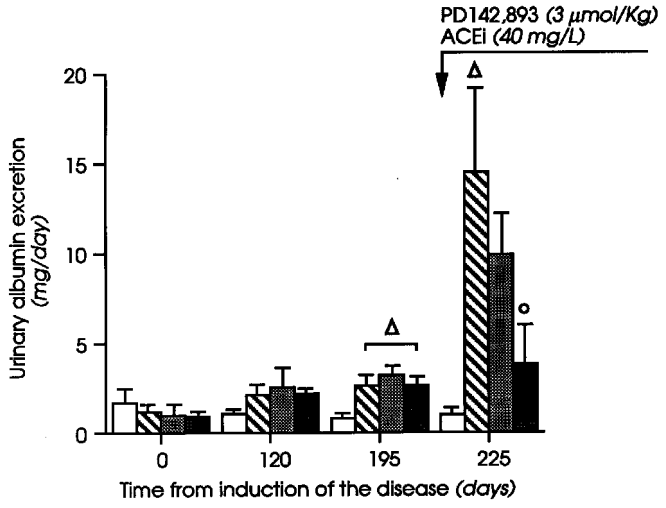

FIG. 4. Urinary albumin excretion in controls (□), diabetic rats (▨), and diabetic rats given PD 142,893 (▩) or lisinopril (■) during the study. Data are means ± SE. Determinations were performed in 8 animals for each group at all time points except at 225 days, when only seven animals in each diabetic group survived. △, $P < 0.05$ vs. controls; ○, $P < 0.05$ vs. diabetics.

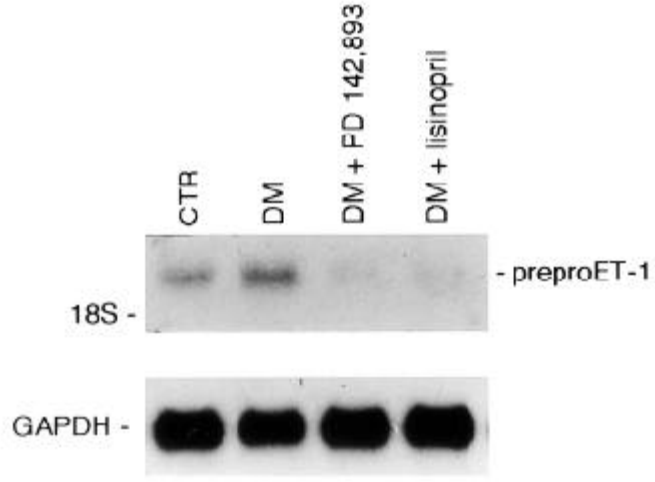

FIG. 5. Renal expression of ET-1 mRNA in diabetic and control rats and effect of treatment with lisinopril or PD 142,893. mRNA samples (10 μg) obtained from pooled kidneys of each experimental group were blotted onto synthetic membranes, which were hybridized sequentially with α^{32}P-labeled rat ET-1 (top) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; bottom) cDNA probes. The optical density of the autoradiographic signals was quantitated and calculated as the ratio of ET-1 to GAPDH mRNA. The mRNA levels were calculated by assuming the optical density of controls as unit. Representative Northern blot analysis of $n = 3$ experiments.

bution in kidneys of untreated or treated diabetic rats with respect to normal rats of the same age. As shown in Fig. 6*A*, in control rats hybridization signal was detectable mainly in epithelial cells of distal tubules. In diabetic rats, ET-1 mRNA staining increased dramatically over controls, and the signal was now detectable in the glomerulus. Strong cytoplasmic staining was also observed in the distal tubules (Fig. 6*B*). The pattern of distribution of renal ET-1 mRNA was remarkably reduced by PD 142,893 or lisinopril, particularly at the glomerular level (Figs. 6*C* and 6*D*, respectively). No hybridization signal could be observed with the ET-1 sense probe (Fig. 6*E*).

Renal function. The results of GFR and RPF are reported in Table 1. Untreated diabetic rats had GFR comparable to controls, but a significantly lower RPF ($P < 0.05$). PD 142,893 and lisinopril increased RPF to values that were not significantly different from those of controls.

Renal histology. Because diabetic rats in the present study were evaluated early in the course of the disease, they had no glomerular lesions and mild tubulo-interstitial inflammation. Histological assessment of kidney specimens from rats given the ET receptor antagonist or the ACE inhibitor indicated some degree of reduction in the signs of tubulo-interstitial damage, which could be measured by a semiquantitative scoring of the lesions (data not shown).

DISCUSSION

Available in vitro data indicate that high glucose concentrations enhance ET synthesis (22) and modulate ET-1 receptors, at least in glomerular mesangial cells (23). In vivo (24) ET-1 mRNA levels increased in glomeruli from rats with streptozotocin-induced diabetes, while ET_A and ET_B receptor expression was unchanged. Higher-than-normal expression of the ET-1 gene in diabetes was normalized by insulin, suggesting that the abnormality was caused, at least in part, by hyperglycemia (24). A logical implication of the above findings is that in diabetic renal disease, ET-1 synthesized in excessive amounts by renal cells may contribute to renal damage by at least two distinct pathways of injury, one mediated by cortical and inner medulla hypoperfusion (given the extraordinary sensitivity of renal vessels [25] to the vasoconstrictor effect of ETs) and the other based on the effect of ET-1 of enhancing extracapillary matrix deposition (26). Data that diabetic rats preventively given an ET_A receptor antagonist, unlike untreated diabetics, were protected from the upregulation of extracellular matrix proteins and growth factor renal genes support a role for ET-1 in renal injury. However, so far there are no true therapeutic studies that could be used to establish whether ET-1 antagonist can cure overt diabetic renal disease. The present study has been designed to clarify the functional relevance of increased ET-1 gene expression in diabetes, with the final aim of establishing whether the consequences of excessive renal ET-1 could possibly be pharmacologically modulated for a long-term benefit on the evolution of the disease. Biological activity of ETs was blocked by unselectively inhibiting the two main receptors, A and B. The effect of inhibiting ET receptors was compared with ACE inhibition, a maneuver that effectively limits proteinuria and prevents renal lesions in diabetic animals and humans as documented consistently by many studies (11,12). Our results indicated that the nonselective ET receptor antagonist PD 142,893, given to diabetic rats with hypertension, overt nephropathy, and proteinuria, normalized systemic blood pressure and reduced urinary proteins. In addition, the ET-1 antagonist ameliorated renal blood flow, which was significantly lower than normal in untreated diabetics. The fact that the ET receptor antagonist also remarkably reduced ET-1 mRNA at both the glomerular and tubular levels supports the possibility of an autoinduction

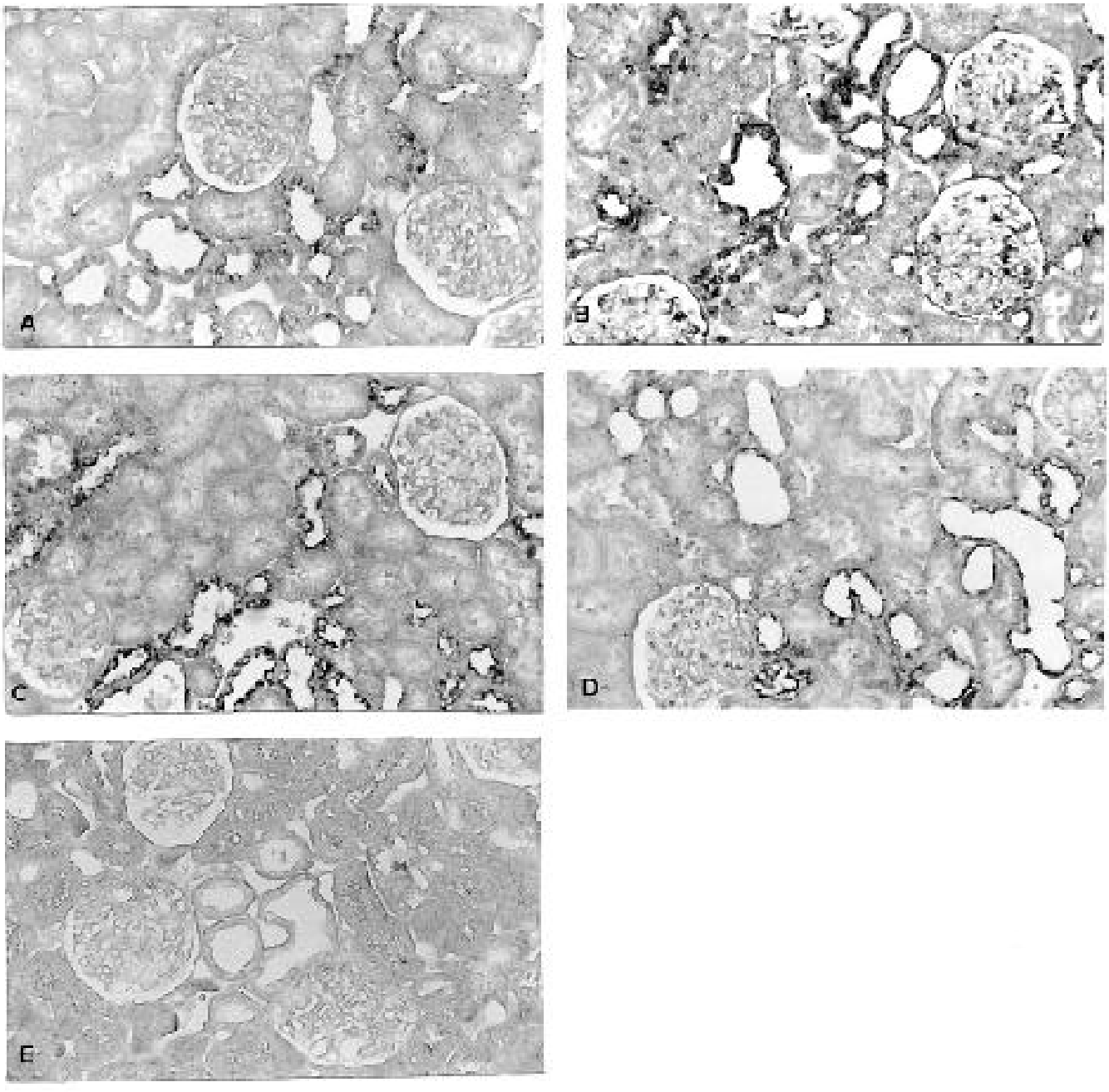

FIG. 6. Representative photomicrographs of the distribution of ET-1 mRNA by in situ hybridization in kidney sections from a control rat (*A*) and from diabetic rats given vehicle (*B*), PD 142,893 (*C*), or lisinopril (*D*). Experiment was repeated in three of seven animals in each group. *A*: Signal present in distal tubules; *B:* signal detectable in glomeruli and stronger in distal tubules. *C* and *D:* Signals reduced particularly in glomeruli; *E:* negative control (ET-1 sense probe). Original magnification ×180.

of ET-1 via the ET_B receptor that was documented recently in rat mesangial cells (27) as well as in human endothelial and proximal tubular cells (28,29).

Compared with PD 142,893, the ACE inhibitor lisinopril was always superior in lowering systemic blood pressure at both time points considered. As observed in previous studies (11,17), we found here that blood pressure in the lisinopril-treated diabetics was significantly lower than in normal controls of the same age. Results of renal function studies did not reveal changes in GFR (whose values are normal at this stage of diabetes) induced by either treatment. Instead, renal blood

TABLE 1
Effects of a chronic administration of PD 142,893 or lisinopril on GFR and RPF in rats with diabetes

Groups	GFR (ml/min)	RPF (ml/min)
Controls	2.21 ± 0.17	7.22 ± 1.17
Diabetic	2.19 ± 0.14	5.64 ± 0.36*
Diabetic + PD 142,893	2.09 ± 0.16	6.24 ± 0.58
Diabetic + lisinopril	2.32 ± 0.23	6.10 ± 0.94

Data are means ± SD. *$P < 0.05$ vs. control.

flow was comparably ameliorated by lisinopril, which also showed similar antiproteinuric properties.

How can this latter finding be interpreted on the basis of the current theories linking perturbation of barrier function to changes in glomerular hemodynamics (30)? Results from animal experiments have consistently documented that in diabetic nephropathy, the relative loss of glomerular autoregulation of flow exposes the capillary tuft to systemic blood pressure. The consequent increase in glomerular capillary pressure with time imposes a mechanic and possibly inflammatory stress on the membrane so as to perturb barrier function first and ultimately impair the anatomical integrity of the capillary network, eventuating in focal or global sclerosis of the tuft (31). Such negative effects on barrier function can be reversed by ACE inhibitors, a class of molecules that by lowering the tone of the efferent arteriole effectively enhances the glomerular flow and restores to normal glomerular pressure and transcapillary pressure gradient (11). As a consequence, filtered protein load is reduced and, in the long run, structural damage to the glomerulus is remarkably limited (32). Findings that an ET-1 receptor antagonist and an ACE inhibitor share comparable antiproteinuric properties could possibly imply that either drug in this model restores glomerular pressure to normal, despite the superior systemic antihypertensive effect of the ACE inhibitor. The concerted action of the ET-1 antagonist we have used here on glomerular arteriolar tone and the ultrafiltration coefficient associated with the effect on systemic blood pressure are conceivably responsible for the improved glomerular permeability in experimental diabetes. An alternative possibility to explain the comparable effect on proteinuria of the two molecules derives from data that ACE inhibitors restore to normal the upregulated ET-1 gene of diabetic rat glomeruli (33). It is therefore possible that perturbations of glomerular barrier function of experimental diabetes recognize a common ET-1–dependent pathway whose cellular and molecular determinants deserve to be further explored.

In conclusion, we have found that an unselective ET antagonist effectively reduced the clinical expression of diabetic nephropathy and ameliorated renal perfusion even when the treatment was initiated after 6 months of diabetes, when the animals already had clinical nephropathy and overt proteinuria. These results support the possibility, raised by many experimental and human studies, that ET-1 is at least one contributory mediator of kidney damage in diabetes, and they open theoretical perspectives for innovative approaches in the treatment of this disease, possibly based on a new class of renoprotective agents.

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