Upregulation of Osteopontin Expression in Renal Cortex of Streptozotocin-Induced Diabetic Rats Is Mediated by Bradykinin

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The model of streptozotocin (STZ)-induced diabetes in Wistar rats was used to study the expression of osteopontin during development of diabetic nephropathy. Diabetes was confirmed by serum glucose levels exceeding 16 mmol/l during the experimental period of 12 weeks. During this period of time, diabetic nephropathy developed, as characterized by a reduced glomerular filtration rate (2.7 ± 0.3 ml/min in controls vs. 1.7 ± 0.1 ml/min in diabetic rats) and proteinuria (8.3 ± 1.7 mg/24 h in controls vs. 22.0 ± 4 mg/24 h in diabetic rats). Northern blot analysis revealed a time-dependent upregulation of renal cortical osteopontin expression reaching 138 ± 6% of control levels after 2 weeks and 290 ± 30% (mean ± SE, *n* = 6–9) after 12 weeks. By immunostaining, the increased osteopontin expression could be located to the tubular epithelium of the renal cortex. Chronic treatment of animals with ramipril (3 mg/kg) during the 12-week experimental period led to a further increase in osteopontin mRNA expression in diabetic animals, amounting to 570 ± 73% (mean ± SE, *n* = 6) of controls. Increased levels of osteopontin were not associated with accumulation of monocyte/macrophages that were identified by the cell type specific monoclonal antibody ED-1. The increased osteopontin expression in ramipril-pretreated rats was abolished by application of the bradykinin B_2-receptor antagonist, icatibant (0.5 mg/kg). In addition, increased osteopontin expression in diabetic rats, which did not receive any treatment after STZ injection, could as well be reduced by icatibant given for the final 2 weeks of the experimental period. These data suggest that a strong bradykinin B_2-receptor–mediated upregulation of osteopontin occurs during the pathogenesis of experimental diabetic nephropathy in rats. *Diabetes* **47:1512–1518, 1998**

The development of renal dysfunction on the basis of insulin-dependent diabetes is the most common cause of end-stage renal disease (1–3). In addition to normalizing serum glucose levels, substituting insulin, and controlling blood pressure, ACE inhibitor treatment has been shown to be effective in delaying the development of diabetic nephropathy (4–7). Besides hyperglycemia, which is the primary metabolic disorder, a variety of mechanisms are believed to be involved in the pathogenesis of diabetic nephropathy. It has been shown that accumulation of extracellular matrix components occurs in diabetic nephropathy, leading to thickening of basement membranes and expansion of glomerular mesangial and tubulointerstitial compartments (8,9). Notably, it is the tubulointerstitial disease that correlates best with the degree of renal impairment and the risk to end-stage renal failure in this and other renal nephropathies (10).

In the present study, we examined the expression of osteopontin during development of diabetic nephropathy, since a growing body of evidence indicates that osteopontin plays a role in various models of acute and chronic diseases, including atherosclerosis (11–13), myocardial necrosis (14), and renal tubulointerstitial fibrosis (15,16). Osteopontin is an arginine-glycine-aspartate (RGD)-containing adhesive glycoprotein (17) that is expressed in a variety of organs, including bone (18,19), kidney (15,16,20), vasculature (21,11), and epithelia (22). Recently, several studies have suggested that osteopontin might play both a pro-inflammatory and protective role in the kidney (23–25).

The aims of the present study were *1*) to test the hypothesis that osteopontin expression is upregulated in experimental diabetic nephropathy and *2*) to obtain information about the regulation of osteopontin expression during the pathogenesis of diabetic nephropathy. Since a role for the angiotensin system in the control of osteopontin expression has been proposed, our approach was to chronically treat streptozotocin (STZ)-diabetic rats with an ACE inhibitor (ramipril). In addition, because ACE inhibitors not only prevent conversion of angiotensin I to angiotensin II, but also inhibit the degradation of kinins, the effect of the specific bradykinin receptor B_2-antagonist, icatibant (Hoe 140) (26), was determined. We found that osteopontin expression was upregulated in diabetic nephropathy and provide evidence that B_2-receptor–mediated events are responsible for this upregulation.

RESEARCH DESIGN AND METHODS

Animal experiments. Conscious male Wistar rats (300–330 g) obtained from K. Thomae (Biberach/Riss, Germany) were used in all experiments. Diabetes was induced by a single intraperitoneal injection of STZ (70 mg/kg; Sigma, Munich, Germany) dissolved in 0.4 ml sodium citrate buffer (0.1 mol/l, pH 4.5). Control rats were injected with equivalent doses of sodium citrate buffer. Damage of pancreatic β-cells was confirmed 24 h after STZ injection by determination of serum glucose levels. Animals with serum glucose levels higher than 16 mmol/l were included in this study. STZ-diabetic rats were pair-fed with an age-matched control group, since STZ-diabetic rats have up to 1.5-fold of an increase of food intake. The animals were kept on a 12-h light/dark cycle and had free access to drinking water.

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BSA, bovine serum albumin; GAPDH, glyceraldehyde-3-phosphate dehyrogenase; GFR, glomerular filtration rate; IgG, immunoglobulin G; PBS, phosphate-buffered saline; SSC, sodium chloride–sodium citrate; STZ, streptozotocin.

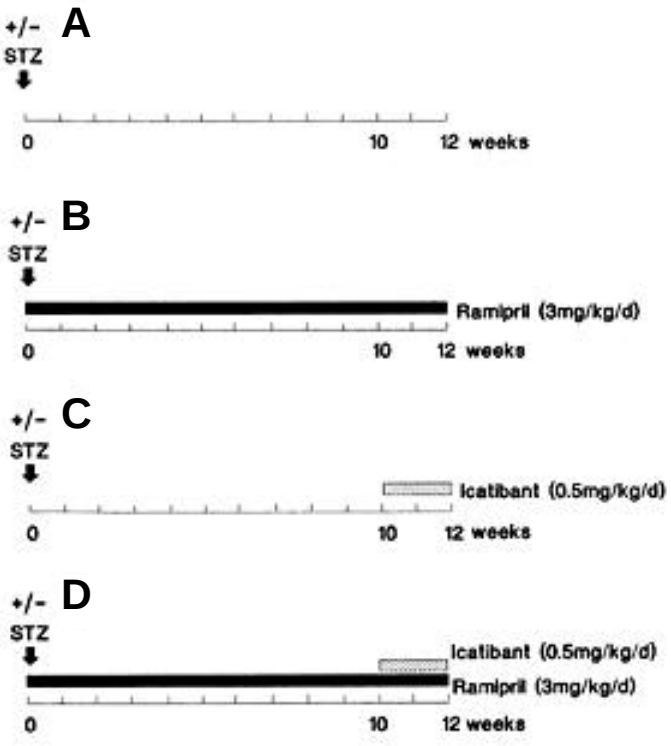

FIG. 1. Diagram showing the eight different treatment groups: buffer and STZ-injected animals serving as control groups for the treated nondiabetic and STZ-diabetic rats during an experimental period of 12 weeks (*A*); nondiabetic and STZ-diabetic animals receiving the ACE inhibitor ramipril (3 mg · kg^{-1} · day^{-1}) for the whole experimental period of 12 weeks (*B*); the 2-week treatment with the bradykinin B_2-receptor antagonist, icatibant (0.5 mg · kg^{-1} · day^{-1}) beginning 10 weeks after STZ injection (*C*); combined treatment with ramipril (12 weeks) and additionally with icatibant during the last 2 weeks (*D*).

The Wistar rats were treated with a subantihypertensive dose of the ACE inhibitor, ramipril, and with the specific B_2-receptor antagonist, icatibant (Hoe 140). Both substances were kindly provided by Hoechst AG (Frankfurt, Germany). Ramipril was administered per gavage as a daily dose of 3 mg/kg during the whole experimental period of 12 weeks. Treatment with the B_2-antagonist was performed 10 weeks after induction of diabetes by implanting an osmotic pump (alzet 2002; Alza Corporation, Palo Alto, CA) subcutaneously in the neck, liberating 500 µg icatibant · kg^{-1} · day^{-1} during 2 weeks. In addition, the effect of treatment with a combination of ramipril and icatibant was evaluated in STZ-injected rats and in control rats. For controls, nondiabetic rats received identical treatment as diabetic rats. Each of the different groups was started with nine animals. The different treatment regimes are depicted in Fig. 1.

Plasma glucose levels were determined every 2 weeks using a reflectance meter (Acutrend; Boehringer-Mannheim, Mannheim, Germany). After 10 weeks, rats were placed individually in metabolic cages, and 24-h urine collections were obtained. Aliquots were stored at –20°C until measurement of renal protein excretion by the pyrogallol-red method (Analyticon, Burbach, Germany). Subsequently, femoral arterial (PP10) and venous (PP25) polyethylene catheters were inserted under chloralhydrate anesthesia. After a 24-h recovery, a glomerular filtration rate (GFR) was measured in conscious rats by single inulin injection method (800 mg/kg) (27). Finally, mean arterial blood pressure was recorded in conscious rats with a Statham P23 Dc pressure transducer connected to a Gould Brush 2400 recorder. Six control and six diabetic animals were killed after 2 weeks. The other groups, consisting of either nondiabetic or diabetic rats, were treated with ramipril, icatibant, or ramipril plus icatibant as described above, and sacrificed at 12 weeks.

Northern blot analysis. After removal of the kidneys, renal cortex was macroscopically separated from the medulla and then frozen in liquid nitrogen and stored at –80°C. After homogenizing the tissue samples, total RNA was extracted using the Trizol reagent (Life Technologies, Gaithersburg, MD) following the manufacturer's instructions. For each blot, 20 µg total RNA were loaded per lane and electrophoresed on a 0.8% agarose gel containing formaldeyde, transferred to Hybond-N membranes (Amersham, Arlington Heights, IL), and immobilized by baking for 2 h at 80°C. For Northern blotting, the ^{32}P-labeled 2B7 cDNA probe (20) for rat osteopontin and a probe for rat glyceraldehyde-3-phosphate dehyrogenase (GAPDH) were used. Hybridization was performed using QuikHyb-hybridization solution (Stratagene, La Jolla, CA), according to the standard protocol. The blots were washed two times for 15 min at room temperature in a low stringency buffer (2 × sodium chloride–sodium citrate [SSC], 0.1% sodium dodecyl sulfate), followed by a 30-min wash in a high stringency buffer (0.1 × SSC, 0.1% sodium dodecyl sulfate) at 65°C. Autoradiography was performed at –70°C with a Kodak biomax MR film. A CAMAG TLC II scanner was used for densitometry. The values obtained for GAPDH by means of densitometry were used to correct data with respect to RNA loading.

Immunochemistry. For osteopontin immunostaining, purified immunoglobulin (IgG) fractions of OP199, a goat polyclonal antibody raised against rat osteopontin (28), and normal goat IgG (control staining) were used. Methyl carnoy-fixed paraffin sections were deparaffinized in xylene (three times), hydrated, and treated with 3% hydrogen peroxide in methanol for 30 min. Nonspecific binding was blocked by incubation with 2% normal rabbit serum in 1% bovine serum albumin (BSA)/phosphate-buffered saline (PBS). All subsequent antibodies were diluted into 1% BSA/PBS. OP199 and normal goat IgG were incubated with specimens for 1 h at room temperature at 20 µg/ml final concentration. After washing, biotinylated rabbit anti-goat IgG (1:4,000, Vector Laboratories, Burlingame, CA) was applied. Subsequently, tissue was peroxidase labeled with an avidin-peroxidase conjugate (ABC Elite, Vector Laboratories). Antigen was visualized by incubation with the substrate 3,3′-diaminobenzidine (Sigma, St. Louis, MO). In addition, immunostaining for monocyte/macrophages was performed with the murine monoclonal antibody, ED-1 (29). A normal mouse IgG was used as control. ED-1 (Bioproducts for Science, Indianapolis, IN) staining was detected with a biotinylated anti-mouse IgG as described above for OP199.

Statistical analysis. Data were analyzed by the one-way analysis of variance (ANOVA). Subsequently, it was tested for significant differences between the means of particular pairs of groups by Bonferroni's *t* test.

RESULTS

Diabetic nephropathy. Within 24 h, STZ treatment induced hyperglycemia (serum glucose concentration ≥16 mmol/l). During the following 12 weeks, plasma glucose levels further increased to ≥28 mmol/l. Levels of serum glucose did not respond to ramipril, icatibant, or the combined treatment. In addition, mean arterial blood pressure was not affected by any drug treatment (Table 1). At the end of the experimental period, GFR and urinary protein excretion of control animals were 2.7 ± 0.3 ml/min and 8.3 ± 1.7 mg/24 h, respectively. Development of diabetic nephropathy was confirmed 12 weeks after STZ injection by reduced GFR (1.7 ± 0.1 ml/min) and by proteinuria (22.0 ± 4.0 mg/24 h). Treatment of diabetic animals with ramipril reduced proteinuria to control levels, whereas additional treatment with the bradykinin antagonist, Hoe 140, did not affect urine protein levels statistically significant. However, there seems to be a trend toward an increase in protein levels in ramipril-treated diabetic rats upon blockade of bradykinin B_2-receptors, suggesting that kinins mediate partially the antiproteinuric action of ramipril. Involvement of kinins in the antiproteinuric action of ACE inhibitors has also been demonstrated in certain animal models by others (30).

Increased osteopontin mRNA and protein expression in renal cortex of diabetic rats. Northern blot analysis revealed weak basal osteopontin expression in the renal cortex of control animals after 2 weeks (data not shown) and 12 weeks. Immunostaining of osteopontin showed the typical distribution pattern in the renal cortex of control animals (Fig. 3*A* and *B*), which has been described previously (15). Osteopontin was expressed in parietal epithelial cells of Bowman's capsule and rarely in the tubular epithelium (Fig. 3*A* and *B*). In diabetic animals, the osteopontin mRNA levels were found to be elevated. This increase was detected as early as 2 weeks after STZ injection (138 ± 6% of controls, $n = 9$) and proceeded during the following 10 weeks of diabetes (Figs. 2 and 3). Quantification of the autoradiographic

TABLE 1
Variables measured after 12 weeks of STZ-induced diabetes

	Normal rats				STZ-diabetic rats			
	Control	Ramipril	Hoe 140	Ramipril + Hoe 140	Control	Ramipril	Hoe 140	Ramipril + Hoe 140
Serum glucose (mmol/l)	7.05 ± 1.01	7.44 ± 1.05	8.44 ± 1.3	7.21 ± 0.72	28.87 ± 3.62	31.7 ± 3.54	32.75 ± 1.84	32.75 ± 1.84
Urine volume (mmol/l)	13.3 ± 2.6	16.5 ± 6.2	13.7 ± 2.1	13.7 ± 4.1	91.6 ± 16.7	97.6 ± 22.2	114.6 ± 14.0	114.7 ± 12.4
GFR (ml/min)	2.7 ± 0.3	2.9 ± 0.3	2.9 ± 0.3	2.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.3	1.8 ± 0.1	1.5 ± 0.2
Urine protein (mg/24 h)	8.3 ± 1.7	8.3 ± 1.7	7.3 ± 2.0	6.1 ± 1.8	22.0 ± 4.0	8.2 ± 2.0*	19.8 ± 3.9 NS	14.6 ± 1.8 NS
Increase in weight (g)	256.7 ± 15.0	189.6 ± 26.0	230.0 ± 8.8	278.1 ± 16.7	37.2 ± 12.6	48.2 ± 17.3	29.4 ± 7.6	32.9 ± 11.2
Mean arterial pressure (mmHg)	92.5 ± 9.9	100.0 ± 10.0	97.1 ± 6.6	96.6 ± 13.4	97.3 ± 7.1	98.2 ± 7.4	99.0 ± 4.5	93.5 ± 11.1

Data are means ± SE. Drug-treated animals received either the ACE inhibitor, ramipril (3 mg · kg^{-1} · day^{-1}), or the bradykinin B_2-receptor antagonist Hoe 140 (icatibant, 0.5 mg · kg^{-1} · day^{-1}) or a combined treatment (ramipril/Hoe 140) as described in METHODS. *P < 0.05 as compared with untreated STZ-diabetic rats; NS, not significantly different from untreated STZ-diabetic rats. The urine protein levels in the diabetic rats treated with ramipril + Hoe 140 did not differ statistically from the ramipril-treated rats if analyzed by one-way ANOVA. The initial body weights were ~320 g.

signals by densitometry revealed that 12 weeks after STZ-injection, osteopontin mRNA levels in the renal cortex were increased about threefold over control levels. The sites in the renal cortex where the elevated osteopontin expression occurred were identified by immunostaining. A strong staining for osteopontin protein was found in tubular epithelial cells of STZ-diabetic rats (Figs. 3C and D). No osteopontin staining was observed in glomerular or interstitial areas. In addition, no staining was observed when normal goat IgG was used instead of anti-osteopontin antibody (data not shown).

Ramipril induces a further increase in osteopontin mRNA levels in diabetic rats. Control and diabetic rats were chronically treated with the ACE inhibitor, ramipril (3 mg · kg^{-1} · day^{-1}), in order to analyze whether angiotensin II or kinins are involved in the regulation of osteopontin expression. This treatment was effective in reducing proteinuria in STZ-injected rats to control levels after 12 weeks, without altering blood pressure, GFR, blood glucose levels, or urine excretion (Table 1). In control animals, ramipril treatment had no effect on osteopontin expression. In contrast, in diabetic animals a further increase of osteopontin mRNA levels was observed after treatment with ramipril (Fig. 2). Quantitative analysis of Northern blots revealed that the osteopontin mRNA levels in renal cortex of diabetic rats doubled (570 ± 73% of nondiabetic controls) in response to ramipril.

After treatment of STZ-diabetic rats with ramipril, osteopontin expression was found mainly in the tubular epithelium of proximal and distal tubules (Fig. 4), as seen for untreated STZ-diabetic rats (Fig. 3) as well. The expression occurred in a focal or patchy pattern with a few osteopontin-positive tubules surrounded by areas of low osteopontin expression. The same distribution pattern was also observed in untreated STZ-diabetic rats.

Immunostaining for monocyte/macrophage. ED-1 staining was performed in order to investigate whether the elevated osteopontin levels in the renal cortex of STZ-diabetic rats (± ramipril) were associated with macrophage accumulation. No accumulation of macrophages was found in osteopontin-positive areas in any treatment group. In contrast, only few macrophages were regularly scattered throughout the cortex (not shown).

Combined treatment with the ACE-inhibitor and the B_2-antagonist. To determine whether angiotensin II or kinins were involved in the upregulation of osteopontin in response to ramipril treatment, animals were treated for 12 weeks

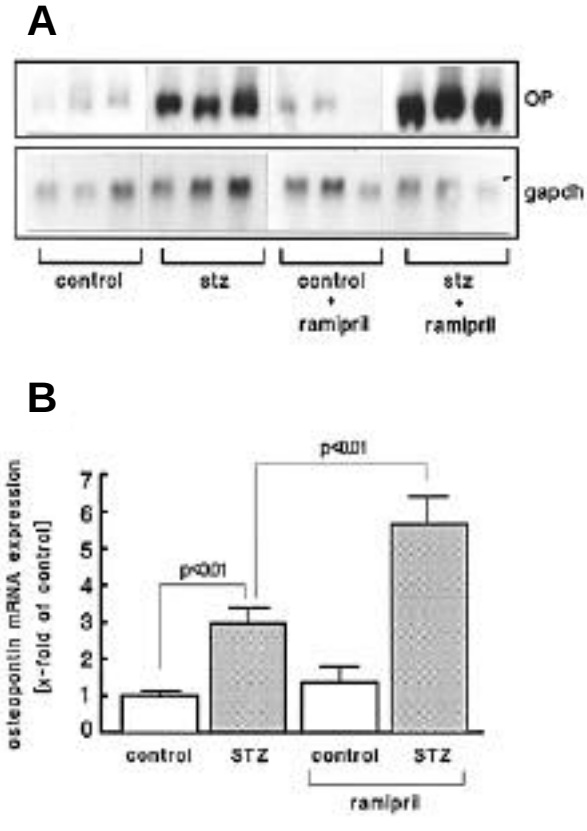

FIG. 2. Effect of ramipril treatment on renal cortical osteopontin expression in normal and diabetic rats at 12 weeks. Figure shows the Northern blot analysis of cortical extracts from normal and diabetic (STZ) rats treated with ramipril (3 mg · kg^{-1} · day^{-1}) (A). In addition to osteopontin, membranes were probed with GAPDH. Quantitative analysis of Northern blots by densitometry (B). Values are corrected for RNA loading using the GAPDH signals and expressed as fold difference compared to controls (n = 6, mean ± SE).

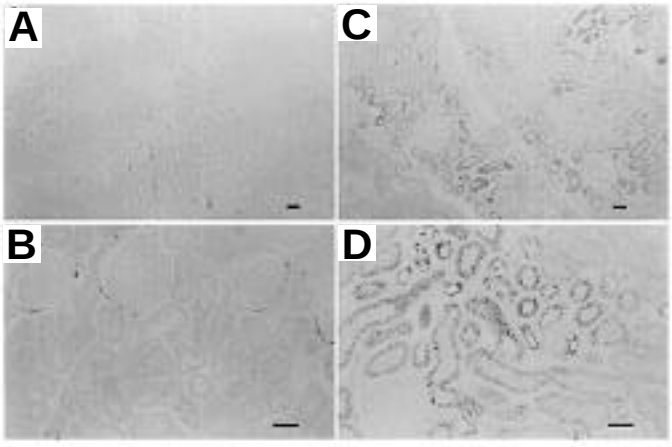

FIG. 3. Osteopontin protein localization in rat renal cortical tissues. Kidney cortices from control (*A*, *B*) and STZ-diabetic rats 12 weeks after induction of diabetes (*C*, *D*) were immunostained using an anti-osteopontin antibody (OP199). No staining was observed when a nonimmune antibody was used as control (not shown). Size bar = 150 µm.

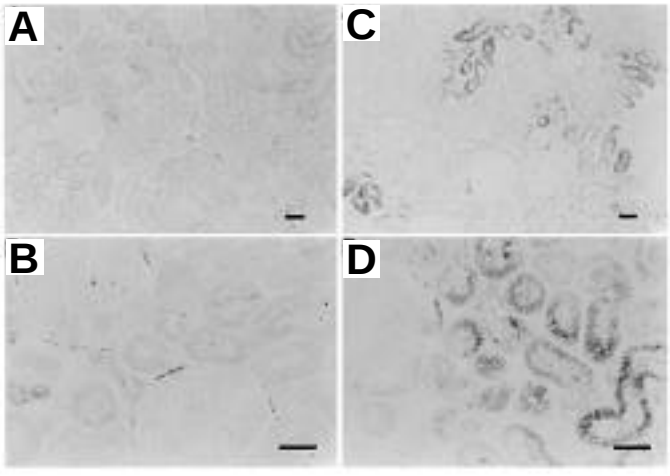

FIG. 4. Osteopontin protein expression in renal cortices of nondiabetic (*A*, *B*) and STZ-diabetic (*C*, *D*) rats after treatment (12 weeks) with ramipril. Immunostaining was performed with the anti-osteopontin antibody OP199. No staining was observed when a nonimmune antibody was used as control (not shown). Size bar = 150 µm.

with ramipril and, in addition, through the last 2 weeks with icatibant (0.5 mg · kg^{-1} · day^{-1}). At the end of this combined treatment, the levels of osteopontin mRNA in diabetic rats were found to be reduced (Fig. 5).

Blockade of B_2-receptors reduces osteopontin mRNA expression in diabetic rats. To investigate whether the upregulation of osteopontin in untreated STZ-diabetic animals was also mediated by bradykinin, rats were treated with icatibant during the 10th through the 12th week of the experimental period. The blockade of bradykinin B_2-receptors reduced the osteopontin expression in the renal cortex of diabetic rats to control levels (Fig. 6), whereas the osteopontin expression in control rats was not affected.

DISCUSSION

We have investigated the regulation of renal cortical osteopontin expression in diabetic nephropathy by evaluating the effects of the ACE inhibitor, ramipril, and the B_2-antagonist, icatibant, in the model of STZ-induced diabetes in rats. In agreement with previous studies (15,31), we found osteopontin protein to be constitutively expressed at low levels in the tubular epithelium and in the parietal epithelial cells of Bowman's capsule in the normal renal cortex. In the present study, a single STZ injection was used for induction of diabetes in male Wistar rats, which led to the development of diabetic nephropathy characterized by reduced GFR and proteinuria. We found a time-dependent upregulation of osteopontin mRNA expression in renal cortical tissue of the diabetic rats, leading to about a threefold increase of osteopontin mRNA expression after 12 weeks. We showed by immunostaining that the upregulation of osteopontin expression occurs mainly in the tubular epithelium of the renal cortex.

Angiotensin II has been suggested to regulate osteopontin levels in the kidney. The evidence for regulation of osteopontin expression by angiotensin II evolved from studies showing that osteopontin is upregulated in angiotensin II–induced tubulointerstitial nephritis (15,32). Angiotensin II also directly activates osteopontin gene expression in vascular smooth muscle cells (11,33). In addition, increased expression of ACE has been found in glomeruli and renal vasculature of STZ-diabetic rats (34). On the other hand, it has recently been shown that angiotensin II does not stimulate osteopontin expression in rat renal epithelial cells (35).

To investigate the influence of angiotensin II on osteopontin expression in experimental diabetic nephropathy, we determined the effect of ACE inhibition on osteopontin expression in renal cortices of diabetic rats. Surprisingly, after chronic treatment with ramipril, osteopontin expression in diabetic animals was further increased, reaching values twofold higher than in untreated diabetic rats and about sixfold higher than in control rats. After treatment with ramipril, osteopontin expression remains mainly in the tubular epithelium of renal cortex, as shown by immunocytochemistry, suggesting that inhibition of the ACE does not activate osteopontin expression in a different cell type as compared to the untreated diabetic animals.

These findings suggest that angiotensin II does not induce osteopontin expression in diabetic nephropathy. On the contrary, our observations raise the question of whether angiotensin II might suppress, or kinins might stimulate, osteopontin expression. This question was of particular interest for us since bradykinin plays an important role in the control of renal circulation, glomerular hemodynamics, and tubular function by regulating vascular tone and prostaglandin synthesis (36–39).

We found that the kallikrein-kinin system is activated in STZ-diabetic rats, as compared with nondiabetic rats characterized by elevated plasma and urinary bradykinin concentrations as well as elevated kininogen and bradykinin B_2-receptor expression (C.T., unpublished observations; 40). To test the potential involvement of kinins in the stimulation of osteopontin expression, the bradykinin receptor B_2-antagonist, icatibant, was given during the last 2 weeks of the experimental protocol either *1*) in combination with ramipril to ramipril-pretreated rats or *2*) given alone to untreated rats. Icatibant was effective in reducing osteopontin expression in both ramipril-pretreated and untreated diabetic rats. These findings suggest that the action of bradykinin via its B_2-recep-

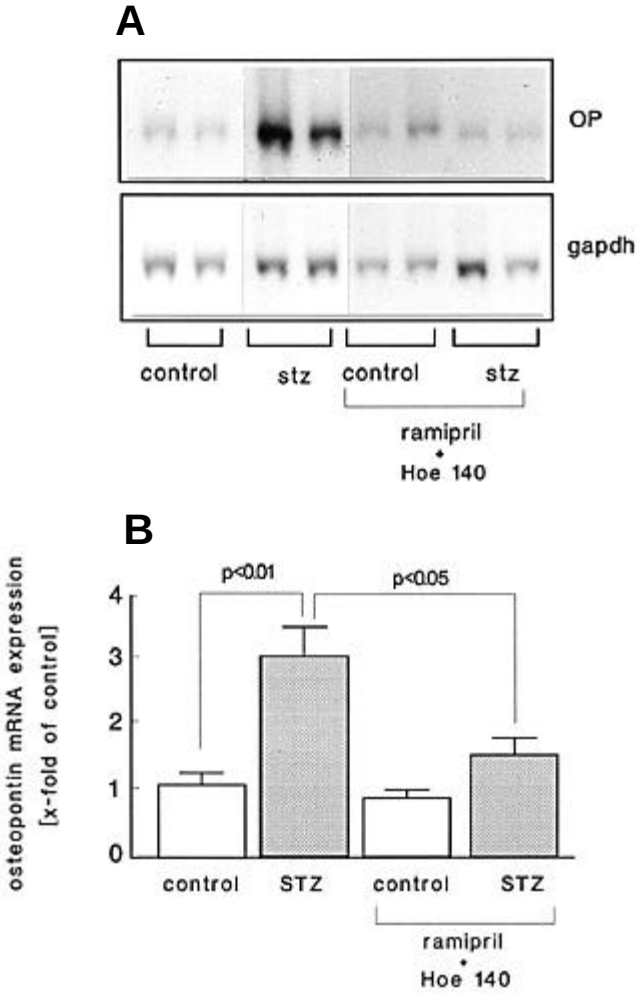

FIG. 5. Osteopontin mRNA expression in kidney cortices after ACE inhibition and additional blockade of bradykinin B_2-receptors. *A*: Northern blot analysis of osteopontin and GAPDH mRNA levels in renal cortical tissue extracts after treatment of animals with ramipril (3 mg · kg^{-1} · day^{-1}, 12 weeks) and icatibant (Hoe 140, 0.5 mg · kg^{-1} · day^{-1}, week 10–12). *B*: Quantitation of osteopontin expression; data were expressed as fold difference compared to controls after normalizing to GAPDH mRNA levels (n = 6, mean ± SE).

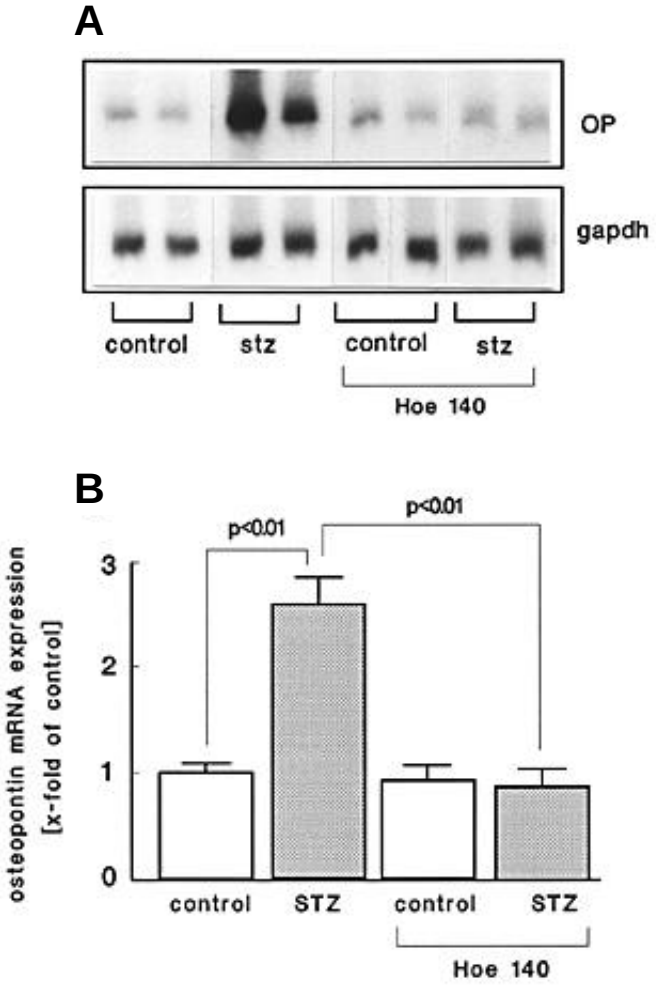

FIG. 6. Influence of bradykinin B_2-receptor blockade on osteopontin mRNA expression in renal cortex. *A*: Representative Northern blots obtained from controls and diabetic (STZ) animals showing the expression of osteopontin versus GAPDH. Animals were treated with the B_2-receptor antagonist icatibant (Hoe 140, 0.5 mg · kg^{-1} · day^{-1}) from week 10–12 after induction of diabetes. *B*: Quantitation of osteopontin mRNA expression by densitometric scanning of autoradiographic signals. Data are shown as fold difference compared to controls after normalizing them to GAPDH mRNA levels (n = 6, mean ± SE).

tor is playing a major role in the regulation of osteopontin expression during the pathogenesis of diabetic nephropathy. Consistent with this mechanism, treatment of diabetic rats with ramipril, which inhibits bradykinin degradation by the ACE, led to a further increase of osteopontin expression, as compared to untreated STZ diabetic rats. In contrast, basal expression of osteopontin under physiological conditions seems to be neither controlled by bradykinin nor by angiotensin II, since drug treatment did not effect osteopontin expression in nondiabetic rats.

The present study does not allow any conclusions as to which signaling pathways might be involved in the bradykinin B_2-receptor–mediated upregulation of osteopontin. The bradykinin B_2-receptor is coupled to G proteins and activates phospholipase C and phospholipase A2 (41,42,46). Phospholipase C activation leads via inositol 1,4,5-trisphosphate to an increase of cytosolic calcium and via diacylglycerol to increased protein kinase C and D activity (43,46). Phospholipase A2 liberates arachidonic acid and leads to increased generation of prostaglandins, for example, that in turn increase cAMP levels and activate protein kinase A (44,45,47,48). In addition, bradykinin increases NO production thus increasing cGMP levels (38,41). It has been described that cAMP and cGMP in certain cell types can regulate the expression of osteopontin (52–55). Furthermore, bradykinin upregulates c*fos* in kidney cells in vitro and increases AP-1 binding activity, which is of interest since the osteopontin promoter contains an AP-1 binding site (49–51). It is likely that the bradykinin B_2-receptor–induced signaling pathways merge and interfere with those of other hormones, cytokines, and growth factors. Clearly, further studies are necessary to uncover the mechanism by which stimulation of bradykinin B_2-receptors increases osteopontin expression in experimental diabetic nephropathy.

The finding that osteopontin was upregulated in the tubular epithelium of renal cortex of diabetic rats is of interest since the diabetic nephropathy is not only a glomerular dis-

ease but is characterized by impaired tubular function as well (10,56). Urinary excretion of low molecular weight proteins and tubular enzymes has been suggested to reflect disturbance and injury of proximal tubules and to proceed microalbuminuria (57,58). Subsequently, during the pathogenesis of diabetic nephropathy, interstitial fibrosis occurs and has been shown to correlate with the development of reduced GFR (59). Since the increased osteopontin expression in the tubular epithelium represents a specific response to bradykinin B_2-receptor stimulation in the diabetic state, it is tempting to speculate that osteopontin might play a role in tubular dysfunction or remodeling. Whether this role is beneficial or detrimental is not known yet (see also below).

Osteopontin has been shown to facilitate macrophage adhesion and migration, and recent studies suggest a role for osteopontin in promoting the accumulation of macrophages. Murine peritoneal macrophages bind with high affinity to osteopontin, and macrophage infiltration occurs at sites of subcutaneous injection of osteopontin in mice (60,61). In addition, colocalization of osteopontin and macrophages occurs in different models of tubulointerstitial injury (15,16). Neutralizing antibodies against osteopontin were shown to be effective in reducing accumulation of macrophages at sites of N-formyl-met-leu-phe injection in mice (24). Neutralizing anti-osteopontin antibodies also reduced renal macrophage accumulation and glomerular injury in experimental crescentic glomerulonephritis in rats (23). Finally, osteopontin knock-out mice show a defective inflammatory response in the kidney after ureteral obstruction (62).

In renal cortex of STZ-diabetic rats, macrophage accumulation was shown to occur at early time points after the induction of diabetes (63). Therefore, it is possible that the upregulation of osteopontin might be involved in an inflammatory response at early time points after induction of diabetes by STZ. However, at later time points (12 weeks), when the highest levels of osteopontin expression were detected, we found in this study no increase in the number of macrophages suggesting that osteopontin has another function in diabetic nephropathy. However, this function has not been uncovered; but, recent evidence suggested that osteopontin might also have beneficial roles under certain circumstances. It has been shown that osteopontin is involved in tissue regeneration and remodeling. With regard to kidney cells, Hwang et al. showed that osteopontin inhibits inducible nitric oxide synthase (iNOS) induction in kidney epithelial cells (64), and Padanilan et al. showed that osteopontin is involved in repair of proximal tubules after ischemic injury (31). In addition, Rogers et al. demonstrated that osteopontin participates in the regulation of nephrogenesis most likely by suppressing tubular apoptosis (25).

It is therefore possible that the elevated tubular osteopontin expression in the renal cortex of STZ-diabetic rats, seen during the advanced stage of the disease, is part of an adaptive repair or regeneration process. In this context it is tempting to speculate that the renoprotective properties of ACE inhibitors might partially relate to the observed effect on osteopontin expression.

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