ACE inhibitor reduces growth factor receptor expression and signaling but also albuminuria through B2-kinin glomerular receptor activation in diabetic rats

Julien Allard, Marie Buléon, Eric Cellier, Isabelle Renaud, Christiane Pecher, Françoise Praddaude, Marc Conti, Ivan Tack, and Jean-Pierre Girolami

Am J Physiol Renal Physiol 293: F1083–F1092, 2007. First published June 27, 2007; doi:10.1152/ajprenal.00401.2006.—Diabetic nephropathy (DN) is associated with increased oxidative stress, overexpression and activation of growth factor receptors, including those for transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF-R), and insulin-like growth factor (IGF-R1). These pathways are believed to represent pathophysiological determinants of DN. Beyond perfect glycemic control, angiotensin-converting enzyme inhibitors (ACEI) are the most efficient treatment to delay glomerulosclerosis. Since their mechanisms of action remain uncertain, we investigated the effect of ACEI on the glomerular expression of these growth factor pathways in a model of streptozotocin-induced diabetes in rats. The early phase of diabetes was found to be associated with an increase in glomerular expression of IGF-I-R, PDGF-R, and TGF-β-R II and activation of IRS1, Erk 1/2, and Smad 2/3. These changes were significantly reduced by ACEI treatment. Furthermore, ACEI stimulated glutathione peroxidase activity, suggesting a protective role against oxidative stress. ACEI decreased ANG II production but also increased bradykinin bioavailability by reducing its degradation. Thus the involvement of the bradykinin pathway was investigated using coadministration of HOE-140, a highly specific nonpeptidic B2-kinin receptor antagonist. Almost all the previously described effects of ACEI were abolished by HOE-140, as was the increase in glutathione peroxidase activity. Moreover, the well-established ability of ACEI to reduce albuminuria was also prevented by HOE-140. Taken together, these data demonstrate that, in the early phase of diabetes, ACEI reverse glomerular overexpression and activation of some critical growth factor pathways and increase protection against oxidative stress and that these effects involve B2-kinin receptor activation.

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The experimental protocol consisted of a prolonged treatment of streptozotocin-induced diabetic rats with ACEI alone or combined with a B2R nonpeptide antagonist. We investigated the effect of this treatment on glomerular expressions of IGF-1, PDGF-B, and TGF-β1 receptors. In addition, we also assessed whether ACE inhibition induced changes in the major activated signaling pathways of these receptors and in the activity of several anti-oxidant enzymes in parallel with microalbuminuria.

MATERIALS AND METHODS

Drugs and compounds. Rabbit polyclonal IgG anti-PDGFR receptor type B, rabbit-purified IgG anti-IRS-1, and antiphospho-Smad 2 were purchased from Upstate Biotechnology (Euromedex, Mundenhollen, France). Goat-purified IgG anti-Smad 2/3, rabbit-purified IgG anti-TGF-β receptor II, and rabbit-purified IgG anti-total form of Erk 2 were from Santa Cruz Biotechnology (Tebu, Le Perray, France). Rabbit-purified IgG anti-phospho-Erk 1 and 2 were purchased from Promega (Madison, WI). The commercial sources of products were otherwise as follows: ornithodanate, N₂,N³-diarginine methyl ester (L-NAME), ouabain, EGTA, SDS, glycerol, PMSF, soybean trypsin inhibitor (SBTI), aprotinin, leupeptin, β-mercaptoethanol, bacitracine, BSA, genitent, DTT, TCA, ammonium molybdate, isobutanol, and toluene were from Sigma (St. Quentin Fallavier, France). NaCl, RPMI 1640, and bromophenol blue were from Merck Eurolab (Strasbourg, France). Tris and glycine were from GIBCO BRL (Cergy-Pontoise, France). PBS was from Biochrom (Berlin, Germany); EDTA was from ICN, and Zanozar (streptozotocin) was from Pharmacia and Upjohn (St. Quentin Yvelines, France). Ramipril and HOE-140 were generously provided by Aventis Pharma (Frankfurt, Germany).

Animal and study design. Male Sprague-Dawley rats (12 wk old, Harlan) were housed under controlled conditions in a room with a 12:12-h light-dark cycle and standard rat chow and tap water available ad libitum. Experimental procedures and protocols were conducted in compliance with the guiding principles for animal research (US) and has been approved by the IFR31 Institutional Animal Care and Use Committee as recently described (3). Diabetes was induced by an intravenous injection of 65 mg/kg streptozotocin (Zanosar, freshly dissolved in saline). The homogenate was centrifuged at 2,300xg for 15 min at 4°C. After neutralization with triocylamine (0.2% vol) and trichlorofluoromethane (0.8 vol), the supernatant was used for determination of malondialdehyde (MDA) after its conjugation to thiobarbituric acid (TBA) and the minimal detection of 1.5 fmol BK with less than 10% cross-reactivity. The expression of B2 receptor in glomerular extract was evaluated by Western blot as previously described (19). The activity of kallikrein in cortical extracts was measured by radioimmunoassay of generated BK after incubation as described below for tissue BK content. To measure BK tissue content, fragments of cortical tissue were rapidly removed and rinsed in PBS containing 0.3 mM orthophenanthroline and 0.3 mM EDTA as kininase inhibitors. They were homogenized in the smallest possible volume of extraction buffer (0.5 ml/100 μg). Then, the homogenates were stored frozen at −80°C until reactivation. BK concentration was measured using a radioimmunoassay as previously described in our laboratory (19). The experimental conditions allowed the minimal detection of 1 fmol BK with less than 10% cross-reactivity with either Des-Arg9-BK or kinnogen. Results are expressed as femtomoles per milligram of protein. ACE activity was measured as previously described and currently performed in laboratory (42).

Statistical analysis. Data are expressed as means ± SE of at least five independent experiments. Body weight, glycemia, and tyrosine phosphatase activity results were analyzed using one-way ANOVA followed by either Student’s t-test for paired data or Dunnett’s test for multiple comparisons. A Kruskal-Wallis test and post hoc Wilcoxon-Mann-Whitney U-test were used for Western blot densitometric...
TABLE 1. Characteristics of control and STZ-diabetic rats without and with treatment by Ins, ACEI, ACEI + HOE, HOE, Ins + ACEI, or Ins + ACEI + HOE

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>No Treatment</th>
<th>Ins</th>
<th>ACEI</th>
<th>ACEI + HOE</th>
<th>HOE</th>
<th>Ins + ACEI</th>
<th>Ins + ACEI + HOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>354 ± 22</td>
<td>238 ± 29*</td>
<td>292 ± 22*</td>
<td>229 ± 37*</td>
<td>241 ± 34*</td>
<td>232 ± 33*</td>
<td>242 ± 28*</td>
<td>247 ± 22*</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.3 ± 0.2</td>
<td>30 ± 6.8*</td>
<td>5.2 ± 0.4</td>
<td>26.8 ± 8.8*</td>
<td>22.1 ± 8.1*</td>
<td>22.9 ± 5.2*</td>
<td>4.9 ± 0.6</td>
<td>5.3 ± 0.4</td>
</tr>
<tr>
<td>Urine volume, ml/24 h</td>
<td>12.8 ± 1.6</td>
<td>150 ± 31.2*</td>
<td>13.6 ± 3.4</td>
<td>116.7 ± 31*</td>
<td>166 ± 21*†</td>
<td>137 ± 22*</td>
<td>11.4 ± 4.1</td>
<td>13.2 ± 2.7</td>
</tr>
<tr>
<td>Creatinine clearance, ml/min</td>
<td>2.53 ± 0.13</td>
<td>1.32 ± 0.27*</td>
<td>1.26 ± 0.28</td>
<td>1.29 ± 0.25*</td>
<td>1.50 ± 0.26*</td>
<td>1.70 ± 0.41*</td>
<td>2.72 ± 0.22</td>
<td>2.61 ± 0.26</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>132 ± 6</td>
<td>141 ± 7</td>
<td>128 ± 5</td>
<td>125 ± 9</td>
<td>142 ± 14</td>
<td>142 ± 11</td>
<td>125 ± 6</td>
<td>129 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE of at least 8 independent experiments. GFR, glomerular filtration rate; STZ, streptozotocin; Ins, insulin; ACEI, angiotensin-converting enzyme inhibitor; ACEI + HOE, ACEI combined with B2-kinin receptor antagonist HOE-140; HOE-140 alone (HOE); Ins + ACEI, Ins combined with ACEI; Ins + ACEI + HOE, Ins combined with ACEI and HOE. *P < 0.05 when compared with nondiabetic control rats. †P < 0.05 when compared with ACEI-treated rats.

RESULTS

Induction of diabetes and effect of treatments. Table 1 and Fig. 1 summarize several physiological parameters of the experimental groups. A single administration of STZ induced significant hyperglycemia, detected 5 days after injection, which was normalized by insulin only but remained unchanged during the other treatments. Induction of diabetes was associated with a large increase in diuresis (150 ± 31.2 vs. 12.8 ± 1.6 ml/24 h for diabetic untreated rats) which was slightly reduced by ACEI treatment via activation of the B2 receptor. Using the tail-cuff method, BP did not significantly vary between the groups. The diabetic untreated group showed a marked reduction of glomerular filtration rate, which was prevented by insulin treatment only. Urinary albumin excretion, shown in Fig. 1, increased significantly from 11.1 ± 1 (control group) to 31.8 ± 3.7 mg/100 mg creatinine (diabetic group); this increase was reversed by insulin or by the ACEI treatment. Interestingly, this effect of the ACEI was abolished when the B2R antagonist HOE-140 was coadministered.

Interestingly, the B2R antagonist alone had no effect per se on any of the parameters tested. Moreover, combining either insulin + ACEI or insulin + ACEI + HOE had no further effect compared with insulin or ACEI given alone.

Effect of treatment with ACEI and B2R antagonist on the glomerular expression of IGF-1, PDGF-B, and TGF-β1 receptors. As shown in Fig. 2, glomerular expression of PDGF-R, IGF1-R, and TGF-β RII was increased in untreated diabetic rats. Treatment with insulin (given after the onset of diabetes) resulted in normalization of glycemia within 3 days but also prevented overexpression of the growth factor receptors. More interestingly, ACEI treatment exhibited similar preventive action despite its lack of effect on hyperglycemia. This effect of ACEI treatment was abolished by coadministration of the B2R antagonist HOE-140. Administration of HOE-140 alone had no significant effect. Moreover, combining either insulin + ACEI or insulin + ACEI + HOE had no further effect compared with insulin or ACEI given alone.

Effect of treatments with ACEI and B2R antagonist on glomerular signaling pathways. Induction of diabetes not only increased some growth factor and cytokine receptors expression, but it also resulted in activation of several signaling pathways as demonstrated by increased phosphorylation of IRS-1, Erk1/2, and Smad 2/3 shown in Fig. 3. Normalization of hyperglycemia with insulin treatment resulted in decreased phosphorylation of these signaling molecules. Treatment with ACEI for 2 wk also partly prevented phosphorylation of IRS-1, Erk1/2, and Smad 2/3. Finally, the ACEI-induced reduction of phosphorylation was blunted by coadministration of the B2R antagonist HOE-140 that did not exhibit any significant effects alone. Moreover, combining either insulin + ACEI or insulin + ACEI + HOE had no further effect compared with insulin or ACEI given alone.

Effect of treatment with ACEI and B2R antagonist on enzymatic antioxidant defense. In a previous report (3), we showed that ACEI reduced lipid peroxidation suggesting a decrease in oxidative stress. Thus, here, we investigated the effect of the various treatments on the enzymatic activities involved in antioxidative defense. Serum MDA was found increased in diabetic rats. This effect was corrected as expected by insulin administration but also by ACEI, an effect that was abolished by HOE. No changes in activities of catalase, SOD Cu/Zn, or SOD were detected in any of the experimental groups (Table 2). In contrast, a significant increase in glutathione peroxidase activity (GPx) was observed in diabetic rats (Fig. 4). Interest-
ingly, ACEI treatment was associated with an additional increase in GPx activity that was completely prevented by coadministration of the B2R antagonist HOE-140 suggesting that activation of the B2R mediates the stimulation of GPx during ACEI treatment.

**Effect of treatment with ACEI and B2R antagonist on tissue kinin content, tissue kallikrein, and ACE activities.** As shown in Fig. 5, the cortical BK content was significantly reduced in diabetic rats. Normalization of ACEI administration partly prevented the decrease in renal cortex BK content which, nevertheless, remained lower than that of control. Coadministration of the B2R antagonist alone or with ACEI was not associated with any specific effects. Moreover, combining either insulin + ACEI or insulin + ACEI + HOE had no further effect compared with insulin or ACEI given alone. Glomerular expression of the B2-kinin receptor assessed by Western blot, shown in Fig. 5A, remained unchanged in diabetic rats and was not affected whatever the treatment. Conversely, a significant reduction of tissue kallikrein activity was observed in cortical extracts of diabetic rats, an effect that was prevented in all the groups receiving insulin, but that was insensitive to any other treatment. ACE activity was significantly reduced in diabetic rats, an effect that was also prevented by insulin. Treatment with ACEI resulted in complete inhibition of ACE activity. Interestingly, no kallikrein and ACE activities could be detected in glomerular extracts.

**DISCUSSION**

The aim of the present study was to clarify the role of B2-kinin receptor activation in the renoprotective effect of ACEI treatment in a type 1 diabetic rat. For this purpose, glomerular activation of some growth factors pathways critically involved in the development of DN was screened. Indeed, an increasing number of reports support the involvement of cytokines and growth factors in the onset and progression of DN (20, 37, 59, 60). Recruitment of these cytokines leads to overexpression of their receptors and activation of their corre-
sponding signaling pathways, such as phosphorylation of IRS-1 and Erk1/2 for IGF-1 and of Smad for TGF-β1. These phenomena are paralleled by the progression of glomerulosclerosis strongly suggesting a link of causality that is sustained by the beneficial effects of their blockades (20, 60, 69). Reducing hyperglycemia with insulin not only reverses both receptor overexpression and activation of these signaling pathways, but it also delays formation of renal lesions. Unfortunately, permanent normoglycemia is hard, if not impossible, to achieve in most diabetic patients. This provides a rationale to resort to a long-term renoprotective treatment independent of glycemic equilibrium. Until now, blockade of the RAS (using ACEI or AT1 receptor antagonists) is the only therapy whose benefit has been clearly demonstrated in humans. The certainty of their protective effects contrasts with the vagueness of their intimate mechanisms of action. It is now clear that the blockade of the
angiotensin pathway by itself is not the only mechanism involved in ACEI-related vascular and renal protection. Downregulation of some critical cytokines, growth factor pathways, and reduction of oxidative stress appear as additional, but main, actions of ACEI treatment (46). The deleterious effects of these cytokines and growth factors on the kidney result from multiple interactions and the identification of a common early step controlling their activation may provide a breakthrough toward a more efficient way to interrupt the cellular mechanisms of glomerular fibrogenesis.

The present study established that, at the critical level of the glomerulus, the renal protective effect of chronic ACEI treatment during diabetes is in fact associated with the downregulation of the glomerular expression of IGF-1, PDGF, and TGF-β1 receptors as well as inhibition of some of their signalings. Although we failed to identify directly the cellular localization of these receptors, it can be suggested that mesangial cells are critically involved in these changes. This suggestion is consistent with numerous previous studies reporting the expression of B2R in cultured mesangial cells (1, 18, 63), whereas the presence of B2R elsewhere in the glomerulus is poorly documented. Less expectedly, our results indicate that these changes critically involve B2R activation since they are largely abolished by coadministration of HOE-140, a specific B2R antagonist.

Among the several growth factors involved in the progression of diabetic glomerulosclerosis, IGF-1 was one of the first and most extensively studied. Several works from Striker’s group (16, 17, 41, 62) indicate that diabetes could be responsible for prolonged activation of the glomerular IGF-1 pathway accounting for the accumulation of extracellular matrix, as a result of an increase in collagen synthesis alongside decreased degradation. An increasing number of reports indicate that blockade of the IGF-1/GH axis is able to prevent or attenuate nephropathy in various experimental models of diabetes. Activation of IGF-1 receptor is likely involved in diabetes-induced glomerular hypertrophy since an IGF-1 receptor antagonist inhibits early renal growth in diabetes (27). Furthermore, somatostatin analogs, which prevent renal IGF-I accumulation, protect the kidney in both type I and type II experimental models of diabetes (26). Finally, the involvement of IGF-1 in the worsening of DN is well accepted but the effects of chronic ACEI treatment on glomerular expression of IGF1-R protein and IGF-1 pathway have not yet been studied.

TGF-β1, which couples the functions of growth factor and inflammatory cytokine, has emerged as a major mediator of renal fibrogenesis (70) playing a central role in the deleterious renal effects of chronic hyperglycemia. In mesangial cells cultured in a high-glucose medium, inhibition of the JAK/STAT signaling pathway, which mediates several TGF-β1 cellular actions, also reduces TGF-β and fibronectin synthesis (70). A new therapeutic approach to control the fibrotic process is based on the inhibition of either the expression or the action of the renal TGF-β pathway (5, 23, 59, 69). Neutralization of

Table 2. Oxidative stress parameters in control and STZ-diabetic rats without and with treatment by Ins, ACEI, ACEI + HOE, HOE, Ins + ACEI, or Ins + ACEI + HOE

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>No Treatment</th>
<th>Ins</th>
<th>ACEI</th>
<th>ACEI + HOE</th>
<th>HOE</th>
<th>Ins + ACEI</th>
<th>Ins + ACEI + HOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase, U/mg protein</td>
<td>39±6.2</td>
<td>38±7.3</td>
<td>39±4.5</td>
<td>38±7.0</td>
<td>42±5.5</td>
<td>40±5.0</td>
<td>37±3.3</td>
<td>41±2.9</td>
</tr>
<tr>
<td>SOD Cu/Zn, U/mg protein</td>
<td>13.8±2.0</td>
<td>14±2.2</td>
<td>14.2±2.4</td>
<td>15±2.4</td>
<td>14.8±2.3</td>
<td>13.9±2.2</td>
<td>12.2±2.7</td>
<td>13.2±2.5</td>
</tr>
<tr>
<td>SOD Mn, U/mg protein</td>
<td>4.1±0.6</td>
<td>3.9±0.6</td>
<td>4.4±0.4</td>
<td>3.8±0.5</td>
<td>4.7±0.5</td>
<td>4±0.8</td>
<td>3.9±0.4</td>
<td>4.1±0.3</td>
</tr>
<tr>
<td>Tissue MDA, U/mg protein</td>
<td>0.74±0.10</td>
<td>0.73±0.14</td>
<td>0.68±0.11</td>
<td>0.67±0.07</td>
<td>0.72±0.11</td>
<td>0.69±0.12</td>
<td>0.71±0.20</td>
<td>0.69±0.40</td>
</tr>
</tbody>
</table>

Values are means ± SE of at least 6 independent experiments. SOD Cu/Zn, Cu/Zn-superoxide dismutase; Mn SOD, Mn-superoxide dismutase; MDA, Malondialdehyde.

Fig. 4. A: plasma malondialdehyde (MDA) levels. B: glutathione peroxidase activity in glomerular extracts of freshly isolated glomeruli from diabetic rats (filled bars) treated with Ins, ACEI, ACEI + HOE, HOE, Ins + ACEI, or Ins + ACEI + HOE. Values are means ± SE of at least 6 independent experiments. *p < 0.05; **p < 0.01, compared with nondiabetic control rats (open bar). †p < 0.05 compared with D.
secreted TGF-β with specific antibodies prevents glomerulosclerosis and renal failure in a model of type 2 diabetes (db/db mice) (5, 70). Interestingly, ACEI treatment decreases renal TGF-β synthesis in several experimental models of diabetes (28) but also in diabetic patients (61).

The pathophysiological role of PDGF-B in the progression of DN is less clear but is under recent evaluation. It has been known for a long time that PDGF is a necessary factor for the normal development of mesangial cells and that knockout mice for PDGF-B receptor lack mesangial cells (39). During diabetes, hyperglycemia is associated with glomerular upregulation of PDGF-B receptor (22, 35) and its activation is likely involved in the progressive development of fibrosis via activation of the TGF-β1 pathway (22).

Reversing established diabetic glomerulopathy remains a fascinating but unsolved therapeutic challenge. With respect to all these previous strategies designed to separately reduce the activation of proliferative and hypertrophic pathways of growth factors, treatment with ACEI shows a considerable advantage because it acts efficiently on all the upstream cytokine/growth factor receptors and pathways suggesting an action on a common very early step, which remains to be identified. It is also interesting to note that ACEI demonstrates an effect similar to that of insulin on the reduction of growth factor expression and signaling activation. However, in contrast to insulin, ACEI has no significant effect on hyperglycemia. Therefore, ACEI acts at a different level than insulin but close to an early effect of hyperglycemia. Early effects of hyperglycemia are 1) activation of protein kinase C and 2) production of glucoxidative products such as Amadori and advanced glycated end products (21) that lead to formation of reactive oxygen species (ROS). The accumulation of ROS in renal tissue in turn activates several signaling pathways associated with fibrogenesis (36).

An increasing number of reports now indicate that ACEI also induce antioxidative effects. The attenuation of oxidative stress by ACEI is mainly interpreted as resulting from the inhibition of the RAS (14). It is true that ANG II is a potent stimulus for superoxide production by activating NAD(P)H oxidases, but surprisingly, the effect of antagonists of ANG II receptor has not yet been as widely tested as ACEI. Based on the current findings, ACEI may act as a “Magic Bullet” against oxidative stress (49) through an additional but unknown mechanism that is independent of their action on BP. Interestingly,
the activation of B2R has never been evoked to account for the antioxidative effect of ACEI. Clearly, in the present study, the effect of ACEI is dependent on activation of the B2-kinin receptor. Although the precise mechanism remains to be elucidated, our report proposes a hypothesis based on an association between activation of the B2R and reduction of oxidative stress. Treatment with ACEI enhanced GPx activity, a well-established anti-oxidant enzymatic system. Moreover, the ACEI-induced increase in GPx activity is significantly blunted by coadministration of the B2R antagonist. Such stimulation or protection of anti-oxidant systems by ACEI inhibitors has been previously documented in various tissues in aging mice and rats (9, 11, 13), in hemodialysis patients (10), and in diabetic rats (12).

How can BK reduce oxidative stress? Activation of B2R results in the formation of nitric oxide (NO), a potent scavenger molecule, which could reduce the accumulation of ROS. However, such a combination of NO and ROS will, in turn, result in a fall in NO availability which may end up in an elevation of BP (51) not observed in our study. We also recently demonstrated that B2R knockout mice exhibit a reduced glomerular capillary surface area and reduced urinary excretion of NO, indicating a tonic effect of B2R in the control of glomerular morphology (57). Finally, a direct effect of BK reducing the oxidative state of rats with acute hyperglycemia (47) has also been reported but to our knowledge no precise mechanism has been demonstrated.

A second interesting observation in our experiment is that ACEI-induced reduction of albuminuria is also mediated by activation of the B2R. Until now, only one study has suggested the involvement of BK in the antiproteinuric effect of ACEI in DN (65), whereas absence of B2R is associated with higher microalbuminuria in diabetes mice (34). It has been demonstrated that DN is markedly enhanced in mice expressing high ACE activity, a situation associated with low levels of kinin and thereby low B2R activation (29). Similar observations have been reported in mice lacking the B2R (34). We also showed that renal fibrosis is increased in B2R null mice in response to unilateral obstruction (58). These three recent reports indicate that absence of B2R worsen DN, thereby suggesting that BK, through activation of the B2R, could exert potent nephroprotective effects. Several BK-dependent mechanisms could account for a reduction of albuminuria. First, it could be through a hemodynamic effect leading to reduction of filtration pressure. It has been reported (34) that induction of diabetes in mice lacking the B2R is associated with increased nephrin and megsin expressions and thereby glomerular hypertrophy. Therefore, BK could protect the steady expression of glomerular proteins such as nephrin, an essential component of the slit diaphragms necessary to maintain selective glomerular filtration, and megsin an inhibitor of matrix protein degradation. Expression of nephrin is increased in early stages of DN and favors loss of functional podocytes. Increasing the level of megsin, an inhibitor of matrix protein degradation, will result in extracellular matrix accumulation.

Whereas the role of the B2R has been poorly studied, the changes in the kallikrein-kinin system (KKS) have been extensively investigated during diabetes, both in humans and in rats. Initial works suggested that renal KKS function is abnormal during diabetes mellitus in patients, an effect related to poor glycemic control (43). Later on, regulation of the renal KKS was extensively investigated in STZ diabetic rats. We found that, whereas B2R renal expression remains stable during diabetes in our model, renal cortex tissue kallikrein activity decreases. This observation is consistent with the fact that in severely hyperglycemic diabetic animals, urinary excretion of kallikrein is reduced, a phenomenon that worsens with time (44). As a consequence, one could predict that during diabetes, renal kinin production in the cortex will be decreased, what we actually documented in renal cortex extract. The observation that tissue kinin content is increased by ACEI is explained by a decrease in kinin degradation rather by an increase in kinin synthesis since we found that ACEI had no effect of tissue kallikrein activity. It was then further suggested that insulin treatment modulates renal kallikrein production, activation, and secretion (31). Several other works from the same group put forward the hypothesis that increased renal production of kinins contributes to diabetes-induced glomerular hyperfiltration (32). More recently, the same group suggested that BK could promote glomerular injury in diabetes (63), an hypothesis which at first could appear opposite to our results but also to those observed in B2R knockout mice (34). However, the conclusion that BK can promote glomerular injury in diabetes was established on the fact that TGF-β1 mRNA, TGF-β RII, CTGF, and B2R expression and levels in renal cortex of diabetic rats increased simultaneously. However, this observation does not establish any causal relationships between these changes. To achieve this point, it should have been interesting to assess the effect of B2R blockade on TGF-β1 mRNA, TGF-β RII, and CTGF. Our present work adds new information indicating that B2-kinin receptor can also exert beneficial effects during the course of DN. We established that a large part of the effects of ACEI, both on the reduction of growth factor receptor expression and signaling but also on albuminuria, is mediated via activation of the B2R receptor because 1) specific pharmacological inhibition of the B2R reduces the effects of ACEI and 2) ACEI largely restored BK generation in cortical tissue. One may question why 50 to 75% restoration of renal cortical BK content during ACEI could be enough to achieve effects similar to those observed normally in rats. In fact, since ACEI clearly blocks ANG II generation, the beneficial action of BK is no longer counterbalanced by the deleterious effects of ANG II. Thus it can be hypothesized that less BK could achieve similar effects to those observed in normal animals with intact ANG II formation capability.

With respect to the beneficial effects of the KKS, it has also been shown that diabetes suppresses kallikrein and renin mRNA gene expression and that these abnormalities are reversed by insulin or IGF-I (33). More recently, the role of the KKS in the physiopathological mechanism of diabetic complications, either nephropathy or cardiomyopathy, has been revisited and new direct mechanisms have been suggested. In this context, BK has been found to be involved in the antiproteinuric effect of ACE inhibition (64).

Finally, kallikrein gene delivery in the rat (48, 60) protects against experimental diabetic cardiopathy. Besides the cardioprotective effect of kinins, the hypothesis of a nephroprotective action of BK via activation of the B2R is also emerging from several works also discussed in this paper. It has been reported that B9972, a new B2R agonist, demonstrates the capacity to reduce both severe pulmonary hypertension and right ventricular hypertrophy but can also induce apoptosis of hyperprolif-
erative cells in precapillary pulmonary arterioles (64). Finally, it has been very recently shown (4) that kinin infusion prevents renal inflammation, apoptosis, and fibrosis via inhibition of oxidative stress and mitogen-activated protein kinase activity in high salt-induced renal lesion in rats.

In summary, we describe a new chronic and potentially beneficial effect of ACEI resulting in the inhibition of glomerular expression of some growth factor receptors and their signaling during the early steps of type 1 diabetes. A critical observation is that almost all the benefits of ACEI are blunted by concomitant and specific B2R blockage. Thus it is likely that BK mediates these effects through B2R activation. The potential protective role of B2-kinin receptor activation during diabetes has been recently reviewed (8) and it is suggested that B2R-specific agonists now merit consideration as a possible new therapeutic approach to protect against DN, an idea consistent with the works of different groups and not solely those involved in the field of DN. The present work suggests that the protective mechanism of B2R activation could be partly mediated by the stimulation of antioxidant defense and by maintaining steady expression of glomerular structural proteins. Moreover, downregulation of several deleterious growth factor pathways is also dependant on B2R activation. These observations and our results finally strengthen the concept of developing B2R agonists to protect the kidney during diabetes and support the evaluation of the renoprotective effect of B2R activation during diabetes, using a specific agonist, independently of ACEI blockade.

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REFERENCES


