Bradykinin reduces growth factor-induced glomerular ERK1/2 phosphorylation

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During diabetic nephropathy (DN), hyperglycemia triggers numerous deleterious biological responses, such as extracellular matrix protein secretion, cell proliferation, and growth factor activation, including IGF-1, PDGF-BB, VEGF, and their receptors (11, 23, 41, 53, 56). These growth factors are suggested to be involved in the hyperplasia and extracellular matrix accumulation associated with acute or chronic glomerulosclerosis (1, 8, 23, 36, 46). The effects of these growth factors are likely occurring via the phosphorylation of the MAPK ERK1/2 (3, 13, 14). Such phosphorylation of this MAPK occurs in the glomerulus and mesangial cells at an early phase of various pathologies such as DN, mesangio proliferative glomerulosclerosis, or high-salt diet-induced nephropathy (5, 9, 30, 31). The ERK1/2 phosphorylation is suggested to play an important role in the establishment of the hyperproliferative state (33, 35). Finally, hyperglycemia-induced MAPK activation can be considered as an early biochemical signaling event, which is the starting point of a deleterious signaling cascade. The upregulation of growth factor activity will emphasize MAPK activation and accelerate the progression of DN. On this basis, control of MAPK activity can be an innovative therapeutic strategy to decelerate the worsening of DN.

The regulation of mitogenic activity by G protein-coupled receptors, particularly regarding the MAPK pathway, has been largely investigated. With respect to the kinin receptors, bradykinin (BK), the agonist of the kinin B2 receptor (B2R), has been shown to induce proliferation in glomerular mesangial cells (6, 20) and fibrosis in vascular smooth muscle cells (18). The profibrogenic effects of BK are associated with the phosphorylation of ERK1/2, which is a prerequisite for the activation of this MAPK (18). Finally, the activation of ERK1/2 by BK has been demonstrated in various cell lines: A431, mesangial, and vascular smooth muscle cells (20, 25, 50).

Whereas the initial studies conducted only with quiescent cells demonstrated mainly mitogenic effects (6, 20), more recent studies by several groups report inhibitions of both cell proliferation and ERK1/2 phosphorylation by BK. Dixon and Dennis (15) evidenced an inhibition of mitogenesis by BK in arterial smooth muscle cells (20, 25, 50). These observations are consistent with a potential therapeutic role of BK and B2R during glomerulosclerosis.

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extend to other growth factors involved in glomerulonephritis.

A substantial amount of experimental and clinical studies have reported that angiotensin-converting enzyme (ACE) inhibitors are renoprotective, notably in DN (29, 38). ACE inhibitors prevent the generation of angiotensin II, which exerts well-known profibrogenic and proliferative effects (54). Nevertheless, ACE inhibitors also favor the accumulation of kinins by preventing their degradation (10). In addition, compelling evidence suggests the involvement of kinins in the renal effects of ACE inhibitors (22, 39), and Tschöpe et al. (48) have shown that kidney B2R is upregulated in streptozotocin (STZ)-diabetic rats. Nevertheless, the roles of BK and B2R in the protective effects of ACE inhibitors during the development of DN remain to be established.

The aim of the present study was threefold: 1) to investigate in freshly isolated rat glomeruli (IG) the effect of B2R activation on the phosphorylation of ERK1/2 induced by IGF-1, PDGF-BB, VEGF and basic (b)FGF; 2) to explore the mechanism involved in the cross talk between BK and growth factors; and 3) to assess whether this cross talk could be pharmacologically recruited in a physiopathological state by exploring the effect of ACE inhibition on the phosphorylation level of glomerular ERK1/2 and on 4-hydroxynonenal (4-HNE) protein derivatization, an index of the oxidative stress in STZ-diabetic rats with regard to the putative involvement of B2R.

**Materials and Methods**

**Animal Use and Care**

Male Sprague-Dawley rats (12 wk old; Harlan; n = 108) 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. Rats were food-starved 18 h before kidneys were collected. Experimental procedures and protocols were ethically approved by the Midi-Pyrenees regional administration in strict compliance with the guiding principles for animal research (US).

**Drugs and Compounds**

The commercial sources of products were as follows. BK, des-Arg9-BK (DBK), IGF-1, PDGF-BB, VEGF, bFGF, orthovanadate, N(2)-nitro-l-arginine methyl ester (l-NAME), ouabain, EGTA, SDS, glycerol, PMSF, soybean trypsin inhibitor (SBTI), aprotinin, leupeptin, β-mercaptoethanol, poly-(Glu-Tyr), bacitracine, BSA, genistein, DTT, TCA, ammonium molybdate, isobutanol, and toluene were from Sigma-Aldrich (St. Quentin Fallavier, France). NaCl, RPMI 1640, thiovantadate, des-Arg9-BK (DBK), IGF-1, PDGF-BB, VEGF, bFGF, l-NAME, ouabain, and the tyrosine phosphatase inhibitor orthovanadate (OV), the incubation was stopped by adding 1 ml of ice-cold PBS containing 1 mM OV. The dose of BK (100 nM) and of the different growth factors was the maximal response dose according to a dose-response curve performed in pilot experiments. Then tubes were centrifuged (15,000 rpm, 4°C, 2 min), and the supernatant was discarded. The pellet containing the glomeruli was resuspended in 100 μl of lysis buffer (10 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 2 mM OV, 0.36 mg/ml PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 0.1% SDS, pH 7.5), sonicated for 10 s, and centrifuged (15,000 rpm, 4°C, 15 min). Insoluble material was discarded, and the proteins of the soluble extract were boiled in Laemmli buffer (32 mM Tris, 1% SDS, 5% glycerol, 0.0005% bromophenol blue, 2.5% β-mercaptoethanol, pH 6.8) for 6 min and stored frozen until SDS-PAGE. Protein concentration was determined by the Bradford protein assay.

**In Vivo Experiments**

Diabetes was induced by an intraperitoneal injection of 65 mg/kg STZ freshly dissolved in 0.05 M citrate buffer, pH 4.5. Age-matched control rats received the vehicle only. Once diabetes was established (4–5 days afterward), diabetic rats were randomly divided into five groups. Rats belonging to the first group received no other treatment. Insulin was given to the rats in the second group as a subcutaneous implant delivering 2 U/24 h (Linsin, Scarborough, ON). Rats in group 3 received 1 mg·kg⁻¹·day⁻¹ of the ACE inhibitor ramipril in their drinking water. Rats from group 4 received ramipril and in addition were subcutaneously injected daily with 0.25 mg/kg of the B2R-selective antagonist HOE-140. Rats from group 5 received 10 mg·kg⁻¹·day⁻¹ of the AT1-receptor antagonist losartan in their drinking water. The selected doses of ramipril (1 mg·kg⁻¹·day⁻¹) and losartan (10 mg·kg⁻¹·day⁻¹) have been previously demonstrated to reverse many functional and morphological events of DN (24, 55). For its part, the dose of HOE-40 (0.25 mg/kg) is twofold that of a dose demonstrated to inhibit the hypotensive effects of BK in vivo (40). Seven days after the initiation of these different treatments, glycemia was measured with a EuroFlash LifeScan glycometer (Issy-les Moulineaux, France), the rats were killed and the kidneys removed for glomerular protein extraction.

**SDS-PAGE and Western Blotting.** Equal amounts of proteins (25 μg) were separated by SDS-PAGE in Tris-glycine buffer under a 150-V, 30-mA current in a Bio-Rad miniature transfer gel apparatus (Mini-Protean, Bio-Rad Laboratories, Richmond, CA) on a 10% SDS-polyacrylamide gel. Proteins were then transferred to a nitrocellulose membrane (Amerham, Orsay, France) in Tris-glycine-methanol buffer under
a 100-V, 300-mA current in a Bio-Rad miniature transfer gel apparatus (Mini-Protein, Bio-Rad Laboratories). The membrane was blotted with the appropriate antibody. Proteins were visualized using a horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin (Amersham) and an enhanced chemiluminescence (ECL) kit (Amersham).

**MAPK phosphorylation, MAPK phosphatase-1 expression, and 4-HNE protein derivatization.** ERK1/2 phosphorylation was assessed by Western blotting with antiphospho-ERK antibodies (dilution 1:3,000; Promega, Madison, WI) that recognize the active forms of ERK1 (molecular wt (MW) = 44 kDa) and ERK2 (MW = 42 kDa). In a preliminary study, we established that under these experimental conditions the detection of ERK1/2 phosphorylation by this method was highly correlated with the incorporation of radioactive phosphorus in the myelin basic protein. Similarly, MAPK phosphatase-1 (MKP-1) expression was studied with an anti-MKP-1 polyclonal antibody (1:250; Santa Cruz Biotechnology, Le Perray, France). 4-HNE protein derivatization was estimated with a clonal antibody (1:250; Santa Cruz Biotechnology, Le Perray, France). 4-HNE protein derivatization was estimated with an antibody that recognizes total ERK1 protein (1:1,500; Santa Cruz Biotechnology, Le Perray, France). 4-HNE protein derivatization was estimated with a clonal antibody (1:250; Santa Cruz Biotechnology, Le Perray, France). 4-HNE protein derivatization was estimated with a clonal antibody (1:250; Santa Cruz Biotechnology, Le Perray, France). 4-HNE protein derivatization was estimated with a clonal antibody (1:250; Santa Cruz Biotechnology, Le Perray, France).

**Tyrosine phosphatase activity.** The poly(Glu-Tyr) substrate was phosphorylated with [32P]ATP as described earlier (42). Glomeruli were isolated and incubated with 100 nM BK for various times as described earlier. After IG lysis, 10 mg of the protein extract were incubated in 500 µl PTP buffer (50 mM Tris·HCl, 0.5 mg/ml bacitracin, 0.1% BSA, 50 mM DTT, pH 7.5) with 30,000 counts/min of [33P]-labeled poly(Glu-Tyr) for 60 min with a ratio of IG of 320% of control value (P < 0.01) and returned to basal levels at 10 min (Fig. 1A, lanes 2–6). IGF-1 (65 nM) also elicited a transient phosphorylation of ERK1/2 that peaked between 2 and 5 min (250% of control P < 0.01) and was no longer detectable at 10 min (Fig. 1B, lanes 8–12). PDGF-BB (8 nM) induced a transient ERK1/2 phosphorylation that peaked at 2 min of incubation (330% of control; P < 0.01) and was back to basal levels at 10 min (Fig. 1C, lanes 14–18). VEGF (25 nM) and bFGF (30 nM) induced an increase in ERK1/2 phosphorylation that peaked at 2 min (P < 0.01) and returned to basal levels at 20 min (Fig. 1, lanes 20–24 and 26–30, respectively). Total ERK1 expression remained unchanged during all these stimulations.

**B2R activation reduced growth factor-induced glomerular ERK1 and -2 phosphorylation.** Next, we studied the effect of a pretreatment with BK and B1-kinin receptor agonist DBK on IGF-1-, PDGF-BB-, VEGF-, and bFGF-induced ERK1/2 phosphorylation. As shown in Fig. 2A, the phosphorylation of ERK1/2 in IG in the presence of IGF-1 was inhibited by BK (lane 5 vs. 4; P < 0.01). This inhibitory effect was not mimicked by DBK (lane 6 vs. 4), which was devoid of any effect by its own (lane 3). Moreover, as shown in Fig. 2, B–D, the phosphorylation of ERK1/2 induced by PDGF-BB, VEGF, and bFGF was also inhibited in the presence of BK (lanes 13 vs. 12, 19 vs. 18, and 25 vs. 24; P < 0.01). This inhibitory effect was not mimicked by DBK (lanes 14 vs. 12, 20 vs. 18, and 26 vs. 24), which was without effect when used alone (lanes 11, 17, and 23). Moreover, incubation with an equimolar concentration of angiotensin II did not reduce IGF-1-induced phosphorylation of ERK1/2 (Fig. 2A, lane 8 vs. 4), whereas angiotensin II did stimulate ERK1/2 phosphorylation when used alone (Fig. 2A, lane 7). In all these experiments, the level of the total form of ERK1 remained unchanged.

**Negative cross talk between B2R and growth factor receptors involves tyrosine phosphatase activation.** The inhibitory effect of BK on IGF-1- or PDGF-BB-induced ERK1/2 activation (Fig. 3, A and B, lanes 4 vs. 3 and 11 vs. 10; P < 0.01) was blocked by pretreatment with the tyrosine phosphatase inhibitor OV (Fig. 3, A and B, lanes 8 vs. 7 and 14 vs. 13). Moreover, similar inhibitory effects of BK were also demonstrated on VEGF- and bFGF-induced ERK1/2 phosphorylation (data not shown). Nevertheless, one could argue that this blockade by OV is in fact related to a nonspecific inhibition of the Na+–K+–ATPase transporter by OV. However, the inhibitory effect of BK on IGF-1-induced ERK1/2 phosphorylation was not blocked by the Na+–K+–ATPase-specific inhibitor ouabain, thus excluding such a hypothesis (Fig. 3C, lane 20 vs. 19). In all these experiments, the level of the total form of ERK1 remained unchanged.

To provide more direct evidence for the involvement of a tyrosine phosphatase, we measured the tyrosine phosphatase activity in glomerular extract after stimulation with BK. The data shown in Fig. 4 demon-
strated that BK induced a time-dependent increase in tyrosine phosphatase activity, reaching a significant 250% maximum increase after 10-min stimulation with 100 nM BK (P < 0.01).

**MKP-1 is not involved in the negative cross talk between B2R and IGF-1R.** Because the induction of the immediate early gene MKP-1 is known to be involved in the reduction of growth factor-induced MAP kinase activation (7), we investigated this possibility by studying the effect of BK on the expression of MKP-1 by Western blot analysis. As shown in Fig. 5, the constitutive expression of MKP-1 was unchanged by incubation in the presence of either IGF-1 (2 min) or BK (20 min) (lanes 2 and 3).

**Nitric oxide synthesis is not involved in the negative cross talk between B2R and IGF-1R.** B2R activation is known to trigger nitric oxide (NO) release from endothelial cells, and NO is known to be antiproliferative (12). However, the prior incubation of IGF with 100 μM L-NAME did not alter the inhibition of IGF-1-induced...
ERK1/2 activation by BK (Fig. 3C, lane 17 vs. 16; P < 0.01), thus excluding any involvement of NO in the negative cross talk between B2R and IGF-1R in IG.

**In Vivo Experiments**

Physiological parameters of STZ-diabetic and control rats. As shown in Table 1, STZ-treated rats exhibited a significant loss of body weight (about -15%; P < 0.01) as well as significant hyperglycemia (336 mg/dl blood glucose; P < 0.01) compared with control rats and thus could be considered as diabetic. Insulin administration normalized blood glucose and body weight (108 ± 16 mg/dl blood glucose and 379 ± 18 g body wt) in rats under ramipril and losartan treatments, but both hyperglycemia and body weight loss persisted.

**B2R activation reduces diabetes-induced glomerular ERK1/2 phosphorylation.** As it can be observed in Fig. 6, STZ-treated rats exhibited glomerular ERK1/2 phosphorylation 280% of control value compared with the untreated control group (lane 2 vs. 1; P < 0.01). Treatment with either insulin or the ACE inhibitor ramipril abolished this activation (lanes 3 and 4 vs. 2; P < 0.01). This inhibitory effect of ramipril was blunted by the blockade of B2R with HOE-140 (lane 5 vs. 4; P < 0.01).
In contrast, treatment with the angiotensin-receptor blocker losartan did not affect the diabetes-induced increase in glomerular ERK1/2 phosphorylation (lane 6). In all the groups of rats, the level of the total form of glomerular ERK1 remained unchanged.

**B2R activation reduces diabetes-induced oxidative stress in glomeruli.** As shown in Fig. 7, STZ-treated rats demonstrated an increased level of 4-HNE protein derivatization, the major increase being observed in the range of 70 kDa compared with untreated control
Such enhancement in 4-HNE protein derivatization is an indication of increased oxidative stress. Treatment with insulin, the angiotensin II-receptor blocker losartan, or the ACE inhibitor ramipril abolished this modification ($P < 0.01; n = 4$). In all groups of rats, the level of the total form of glomerular ERK1 remained unchanged.

**DISCUSSION**

This paper reports for the first time the inhibition by BK of ERK1/2 phosphorylation triggered in rat IG by different growth factors, namely, IGF-1, PDGF-BB, VEGF, and bFGF. This negative cross talk occurs selectively via B2R activation and involves tyrosine phosphatase activity. An increase in total tyrosine phosphatase activity was measured after BK treatment ($P < 0.01; n = 4$).

Table 1. Data for rats included in in vivo experiments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wt Before treatment, g</th>
<th>Glycemia After Treatment, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>370 ± 2</td>
<td>93 ± 9</td>
</tr>
<tr>
<td>STZ</td>
<td>365 ± 9</td>
<td>336 ± 13‡</td>
</tr>
<tr>
<td>STZ + insulin</td>
<td>369 ± 12</td>
<td>108 ± 16</td>
</tr>
<tr>
<td>STZ + ramipril</td>
<td>351 ± 7</td>
<td>335 ± 41‡</td>
</tr>
<tr>
<td>STZ + ramipril + HOE-140</td>
<td>343 ± 7</td>
<td>399 ± 12†</td>
</tr>
<tr>
<td>Losartan</td>
<td>347 ± 13</td>
<td>354 ± 23†</td>
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</tbody>
</table>

Values are means ± SE of 4 rats/group. STZ, streptozotocin. **$P < 0.01$ compared with the weight before treatment. †$P < 0.01$ compared with control.

This inhibitory effect of ramipril was blunted by the blockade of B2R with HOE-140 ($P < 0.01; n = 4$). In all groups of rats, the level of the total form of glomerular ERK1 remained unchanged.
BK reduces ERK1/2 activation

Fig. 7. Effect of B2R blockade on 4-hydroxynonenal (4-HNE) protein derivatives labeling in IG of STZ-diabetic rats. STZ-diabetic rats were either untreated or received insulin (2 U/day), losartan (10 mg·kg−1·day−1), ramipril (1 mg·kg−1·day−1), and/or HOE-140 (0.25 mg·kg−1·day−1). Western blot analysis was performed as described in the legend of Fig. 1 with an antibody directed against 4-HNE Michael protein adducts (n = 4). **P < 0.01 compared with diabetic rats (lane 2). ##P < 0.01 compared with diabetic rats treated with ramipril.

phatase activation. The second original finding is that chronic ACE inhibition reduces the early diabetes-induced glomerular ERK1/2 phosphorylation as well as oxidative stress in vivo through endogenous B2R activation. Hence, the data suggest the involvement of BK in the therapeutic effects of ACE inhibitors during the development of DN and also underscore, during in vivo ACE inhibition, the pharmacological recruitment of the negative cross talk between B2R and growth factor receptors as seen ex vivo.

Our ex vivo observation of the negative modulation by BK of various growth factor signaling is consistent with the inhibition by BK of mesangial cell proliferation (2). This negative cross talk appears to be specific for BK and B2R because angiotensin II, another ligand for a G protein-coupled receptor, is unable to reduce IGF-1-induced ERK1/2 phosphorylation. Graness et al. (26) also observed an inhibition of EGF signaling after tyrosine phosphatase activation by BK in A431 cells. Therefore, the fact that BK can inhibit ERK1/2 phosphorylation triggered by receptors of different growth factors, IGF-1, PDGF-BB, VEGF, bFGF, and EGF, strongly suggests that the inhibitory action of BK occurs at a common downstream level of growth factor signaling, most likely tyrosine phosphatase activation directed on ERK1/2. Such a hypothesis is consistent with our finding of BK-induced tyrosine phosphatase activity in IG. The involved tyrosine phosphatase is unlikely to be MKP-1. Indeed, it is acknowledged that the activity of this early gene product is essentially regulated at its expression level (5, 7). Therefore, our observation that BK does not modify glomerular MKP-1 protein expression argues against such involvement of MKP-1 in the inhibitory effect of BK on growth factor signaling. On the other hand, our group recently demonstrated in vitro that B2R fixation by BK triggers the activation of the protein tyrosine phosphatase SHP-2 via a direct protein-protein interaction resulting in the inhibition of cell proliferation (19). Therefore, SHP-2 is an obvious candidate, although the in vivo demonstration of its involvement in the negative cross talk between BK and growth factors in IG is still hampered by the lack of a specific inhibitor.

The inhibition of growth factor-induced ERK1/2 phosphorylation in IG by BK could be correlated with an inhibition of a downstream effect such as cell proliferation, which is believed to involve ERK1/2 activation. The control of cell proliferation can occur at a distinct level either by reducing mitogenesis or by increasing cell death. The present data favor an inhibitory action on the proliferative pathway. In this respect, another study has shown that BK reduced smooth muscle cell proliferation induced by PDGF via an unknown mechanism (15). Several other studies have demonstrated an antiproliferative effect of BK in different cell lines without any proposed mechanism (2, 43).

On the other hand, contrasting evidence has shown that BK induces proliferation in glomerular mesangial cells (20). Moreover, the activation of ERK1/2 by BK has been demonstrated in various cell lines: A431, mesangial, and vascular smooth muscle cells (20, 25, 50). It is noteworthy that the proliferative effect of BK has been essentially demonstrated in quiescent mesangial cells with high concentrations of BK. Therefore, it can be suggested that the mitogenic action of BK might depend on the level of cell activation by a growth factor. In a starving condition, BK might promote cell proliferation and ECM protein secretion, whereas the opposite effect becomes preferential during proliferation states, such as glomerulosclerosis, after activation by several growth factors.

The inhibition by B2R activation of IGF-1, PDGF-BB, VEGF, and bFGF signaling might be of physiopathological relevance as the involvement of all these growth factors has been evoked in the progression of glomerulosclerosis, notably during DN but also in renal fibrogenesis. IGF-1 increases glucose uptake in mesangial cells by augmenting the expression of GLUT1 (4, 34) and stimulates the secretion of collagen I and IV and proliferation by mesangial cells (14, 21).

However, other evidence contests the existence of a role for IGF-1 in the establishment of DN. Indeed, Doi et al. (17) have shown that transgenic mice overexpressing IGF-1 do not exhibit glomerulosclerosis and tubular atrophy, which are hallmarks of DN. Nevertheless, since that initial work, a larger number of reports suggest a role for growth factors in the progression of DN and thereby may be of interest for the future development of new drugs useful in the treatment of diabetic kidney disease (23). Interestingly, the prolif-
erative activity of IGF-1 is amplified by prior stimulation with PDGF (16). PDGF-BB, secreted by glomerular cells as well as activated platelets and macrophages, is the most potent mitogen for mesangial cells in vitro and in vivo (36). Moreover, PDGF has been shown to be induced by TGF-β and to mediate TGF-β-induced accumulation of collagen IV and fibronectin (32). Also, VEGF has been reported to enhance collagen synthesis via the activation of ERK (3). bFGF was acknowledged to elicit the proliferation of both fibroblasts and mesangial cells (44, 46) and thereby is involved in renal fibrogenesis. Because these four growth factors are involved in the development of DN, it is conceivable that the negative modulation of their signaling by BK may be of therapeutic relevance in DN.

Next, we demonstrated in vivo that the increased phosphorylation of ERK1/2 and oxidative stress assessed by the detection of 4-HNE protein derivatization, a well-established index of oxidative stress in diabetic glomerular lesions (47), in glomeruli of STZ-diabetic rats is reversed by ACE inhibition via B2R activation. This confirms the physiopathological relevance of our ex vivo observations of negative cross talk between BK and growth factor receptors in IG from normal rats. The increased phosphorylation of glomerular ERK1/2 at an early phase of STZ-induced diabetes was also observed by Awazu et al. (5) and Haneda et al. (31). Awazu et al. (5) ascribed the diabetes-induced activation of ERK1/2 to decreased phosphatase activity and MKP-1 protein expression. This hypothesis is further supported by the fact that tyrosine phosphatase inhibition with OV mimics the diabetic phenotype in mesangial cells, i.e., increased cell proliferation, activation of protein kinase C, tyrosine phosphorylation of intracellular proteins, and induction of PDGF-B chain gene expression (52). In addition, we now demonstrate that hyperglycemia plays a primary role in glomerular ERK1/2 phosphorylation and oxidative stress during diabetes because strict glycemic control with insulin abolished MAPK phosphorylation as well as 4-HNE protein derivatization. Early activation of glomerular ERK1/2 in diabetes is suggested to play an important role in the progression of DN (33, 35) and is consistent with a combined effect of high glucose and various growth factors, including IGF-1, PDGF-BB, and VEGF (23, 54). Moreover, oxidative stress may play an important role in the progression of DN and emphasize the phosphorylation of ERK1/2 (27, 28).

One may note that strict glycemic control with insulin, which is obviously the more appropriate initial therapy for type I diabetes, is as efficient as ACE inhibitors to reduce diabetes-induced phosphorylation of ERK1/2 and 4-HNE protein derivatization. However, the diabetes-induced phosphorylation of ERK1/2 and oxidative stress are reversed by ACE inhibition, without any effect on glycemia, suggesting the involvement of a glycemia-independent mechanism. Therefore, strict glycemic control with insulin and ACE inhibitors may exert independent and additive effects and thereby may be successfully associated to delay or stabilize the rate of progression of renal disorder associated with diabetes, as recently recommended (37). Such an effect of ACE inhibition is consistent with the renoprotective effects of this treatment during diabetes mellitus (23, 29, 38). Furthermore, it was shown that ACE inhibition favors the accumulation of BK (10). Hence, according to present evidence, ACE inhibition potentiates BK concentration and reduces phosphorylation of ERK1/2 and oxidative stress through B2R activation, because blockade of the B2R abolished the effect of ACE inhibitors. Moreover, in vitro stimulation of IG from untreated diabetic rats with BK reduced the enhanced ERK1/2 level, confirming the involvement of B2R (data not shown). In contrast to ramipril, losartan did not reduce diabetes-induced ERK1/2 phosphorylation although it reduced 4-HNE protein derivatization.

This result is consistent with the effect of angiotensin II shown in Fig. 2A, in which angiotensin II did not inhibit IGF-1-induced ERK1/2 phosphorylation. Although both ACE inhibitors and AT1 receptor blockade are renoprotective, notably concerning oxidative stress, it seems that the inhibition of growth factor-induced ERK1/2 phosphorylation is specific to BK. The present observation supports the existence of a protective action of BK and the B2R against the deleterious effects of high glucose and growth factors present in the glomeruli during diabetes mellitus. The hypothesis of a tonic-protective role of BK, at least via the B2R, against the severity of renal complication associated with diabetes mellitus is in agreement with a recent report using transgenic mice for ACE (34a). This report demonstrates that modest genetically determined increases in plasma ACE levels, which decrease BK concentration without significantly affecting angiotensin II (45) result in severe renal complications in the diabetic mouse.

In conclusion, the activation of the B2R inhibits IGF-1-, PDGF-BB-, VEGF-, and bFGF-induced ERK1 and -2 phosphorylation in IG from normal rats, via the activation of a tyrosine phosphatase. This negative cross talk could be pharmacologically recruited during ACE inhibition, demonstrating that chronic activation of B2R under such treatment inhibits the phosphorylation of ERK1/2 as well as oxidative stress in glomeruli of STZ-diabetic rats. These inhibitory actions in the glomeruli are consistent with a renoprotective action of BK and B2R during diabetes mellitus and support the existence of a role for this autacoid in the beneficial effects of ACE inhibition during the development of DN. Such findings open new perspectives concerning the treatment of glomerulosclerosis, notably during diabetes mellitus.
REFERENCES