

**Eric Cellier, Marilynne Mage, Johan Duchêne, Christiane Pécher, Réjean Couture, Jean-Loup Bascands and Jean-Pierre Girolami**

*Am J Physiol Renal Physiol* 284:282-292, 2003. First published Oct 1, 2002;  
doi:10.1152/ajprenal.00115.2002

**You might find this additional information useful...**

---

This article cites 55 articles, 30 of which you can access free at:

<http://ajprenal.physiology.org/cgi/content/full/284/2/F282#BIBL>

This article has been cited by 3 other HighWire hosted articles:

**Pharmacological blockade of B2-kinin receptor reduces renal protective effect of angiotensin-converting enzyme inhibition in db/db mice model**

M. Buleon, J. Allard, A. Jaafar, F. Praddaude, Z. Dickson, M.-T. Ranera, C. Pecher, J.-P. Girolami and I. Tack

*Am J Physiol Renal Physiol*, May 1, 2008; 294 (5): F1249-F1256.

[Abstract] [Full Text] [PDF]

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

J. Allard, M. Buleon, E. Cellier, I. Renaud, C. Pecher, F. Praddaude, M. Conti, I. Tack and J.-P. Girolami

*Am J Physiol Renal Physiol*, October 1, 2007; 293 (4): F1083-F1092.

[Abstract] [Full Text] [PDF]

**Thrombin inhibits migration of human hepatic myofibroblasts**

J. Gillibert-Duplantier, V. Neaud, J.-F. Blanc, P. Bioulac-Sage and J. Rosenbaum

*Am J Physiol Gastrointest Liver Physiol*, July 1, 2007; 293 (1): G128-G136.

[Abstract] [Full Text] [PDF]

Updated information and services including high-resolution figures, can be found at:

<http://ajprenal.physiology.org/cgi/content/full/284/2/F282>

Additional material and information about *AJP - Renal Physiology* can be found at:

<http://www.the-aps.org/publications/ajprenal>

---

This information is current as of February 9, 2010 .

# Bradykinin reduces growth factor-induced glomerular ERK1/2 phosphorylation

ERIC CELLIER,<sup>1</sup> MARILYNE MAGE,<sup>1</sup> JOHAN DUCHÊNE,<sup>1</sup> CHRISTIANE PÉCHER,<sup>1</sup>  
RÉJEAN COUTURE,<sup>2</sup> JEAN-LOUP BASCANDS,<sup>1</sup> AND JEAN-PIERRE GIROLAMI<sup>1</sup>

<sup>1</sup>Institut National de la Santé et de la Recherche Médicale U388, IFR 31, Institut  
Louis Bugnard, 31403 Toulouse Cedex 4, France; and <sup>2</sup>Department of Physiology,  
Faculté de Médecine, Université de Montréal, Montreal, Quebec, Canada H3C 3J7

Submitted 25 March 2002; accepted in final form 24 September 2002

**Cellier, Eric, Marilyne Mage, Johan Duchêne, Christiane Pécher, Réjean Couture, Jean-Loup Bascands, and Jean-Pierre Girolami.** Bradykinin reduces growth factor-induced glomerular ERK1/2 phosphorylation. *Am J Physiol Renal Physiol* 284: F282–F292, 2003. First published October 1, 2002; 10.1152/ajprenal.00115.2002.—Several experimental data report both mitogenic and antimitogenic effects of bradykinin (BK). To conciliate these apparent opposite effects, we hypothesized that, depending on cell context activation, BK could reduce the mitogenic effect of growth factors. Therefore, in the present study we assessed the existence of possible negative cross talk between BK and potential pathogenic growth factors in freshly isolated rat glomeruli (IG). Next, we determined whether this cross talk could be pharmacologically recruited during angiotensin-converting enzyme (ACE) inhibition in the diabetic rat. In IG from normal rats, BK, via activation of the B<sub>2</sub> kinin receptor (B<sub>2</sub>R), causes a transient stimulation of ERK1/2 phosphorylation, whereas it inhibits ERK1/2 phosphorylation induced by IGF-1, PDGF-BB, VEGF, or basic FGF. The reduction of growth factor-induced ERK1/2 phosphorylation is abolished by an inhibitor of tyrosine phosphatase. In glomeruli from diabetic rats, hyperglycemia increased the phosphorylation level of ERK-1/2 as well as oxidative stress. The reversal of these events by ACE inhibition is mediated via B<sub>2</sub>R activation. These observations are consistent with a potential therapeutic role of BK and B<sub>2</sub>R during glomerulosclerosis.

kinin B<sub>2</sub> receptor; growth factors; mitogen-activated protein kinases; angiotensin-converting enzyme inhibition

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, mesangioproliferative 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 B<sub>2</sub> receptor (B<sub>2</sub>R), 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 stimulated with PDGF-AB. Our laboratory recently demonstrated that B<sub>2</sub>R activation reduces serum-stimulated mitogenesis in rat mesangial cells (2). Moreover, Graness et al. (26) have shown that EGF-induced ERK1/2 activation was inhibited by BK via tyrosine phosphatase activation, and Tsuchida et al. (49) suggested an antihypertrophic role for B<sub>2</sub>R on the renal vasculature. However, it is not known whether this cross talk prevails in vivo and might

Address for reprint requests and other correspondence: J.-P. Girolami, INSERM U388, IFR 31, Institut Louis Bugnard, Bâtiment L3, CHU Rangueil, 31403 Toulouse cedex 4, France (E-mail: girolami@toulouse.inserm.fr).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

extend to other growth factors involved in glomerulosclerosis.

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 B<sub>2</sub>R is upregulated in streptozotocin (STZ)-diabetic rats. Nevertheless, the roles of BK and B<sub>2</sub>R 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 B<sub>2</sub>R 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 B<sub>2</sub>R.

## 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-Arg<sup>9</sup>-BK (DBK), IGF-1, PDGF-BB, VEGF, bFGF, orthovanadate, *N*<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME), ouabain, EGTA, SDS, glycerol, PMSF, soybean trypsin inhibitor (SBTI), aprotinin, leupeptin,  $\beta$ -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, and bromophenol blue were from Merck Eurolab (Strasbourg, France). Tris and glycine were from GIBCO BRL (Cergy-Pontoise, France). [<sup>32</sup>P]ATP was from Amersham Biosciences (Saclay, France), PBS from Biochrom (Berlin, Germany), EDTA from ICN, and STZ from Pharmacia and Upjohn (St. Quentin Yvelines, France). Ramipril and HOE-140 were generously provided by Aventis Pharma (Frankfurt, Germany), whereas losartan was a kind gift from Merck (Rahway, NJ).

### Ex Vivo Experiments

**Isolated glomerular preparation.** Glomeruli were isolated as routinely performed in the laboratory by graded sieving (6). Briefly, rats were anesthetized intraperitoneally with 65 mg/kg pentobarbital sodium (Sanofi, Montpellier, France) and killed by exsanguination, and the kidneys were quickly removed. The cortex was forced through three consecutive steel sieves with decreasing pore sizes (180, 125, and 75  $\mu$ m) to harvest  $\sim$ 12,000 glomeruli/kidney. Under light microscopy, >90% of the glomeruli appeared to be decapsulated and free of surrounding tubules and arterioles. Glomeruli were resuspended in RPMI 1640 culture medium and redistributed in experimental tubes containing  $\sim$ 5,000 glomeruli/tube. After the appropriate incubation time at 37°C, and in the presence of 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  $\mu$ l of lysis buffer (10 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 2 mM OV, 0.36 mg/ml PMSF, 10  $\mu$ g/ml SBTI, 1  $\mu$ g/ml aprotinin, 1  $\mu$ g/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%  $\beta$ -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 (Linshin, Scarborough, ON). Rats in group 3 received 1 mg·kg<sup>-1</sup>·day<sup>-1</sup> 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 B<sub>2</sub>R-selective antagonist HOE-140. Rats from group 5 received 10 mg·kg<sup>-1</sup>·day<sup>-1</sup> of the AT<sub>1</sub>-receptor antagonist losartan in their drinking water. The selected doses of ramipril (1 mg·kg<sup>-1</sup>·day<sup>-1</sup>) and losartan (10 mg·kg<sup>-1</sup>·day<sup>-1</sup>) 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  $\mu$ g) 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 (Amersham, Orsay, France) in Tris-glycine-methanol buffer under

a 100-V, 300-mA current in a Bio-Rad miniature transfer gel apparatus (Mini-Protean, 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] and ERK2 (MW = 42). 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 (MPK-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 polyclonal antibody directed against 4-HNE Michael adducts (1:2,000; Calbiochem, Nottingham, UK). The amount of total ERK was also visualized as a control using an antibody that recognizes total ERK1 protein (1:1,500; Santa Cruz Biotechnology) independently of its level of phosphorylation.

**Tyrosine phosphatase activity.** The poly(Glu-Tyr) substrate was phosphorylated with [ $^{33}\text{P}$ ]ATP as described earlier (42). Glomeruli were isolated and incubated with 100 nM BK for various times as described earlier. After IG lysis, 10  $\mu\text{g}$  of the protein extract were incubated in 500  $\mu\text{l}$  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  $^{33}\text{P}$ -labeled poly(Glu-Tyr) for 10 min at 30°C. The reaction was stopped by addition of 1 volume of ice-cold 30% TCA and incubated 30 min on ice. After centrifugation at 13,000 rpm for 10 min at 4°C, 1 volume of ammonium molybdate was added to 1 volume of supernatant, and the mixture was incubated for 10 min at 30°C. Then, 2 volumes of isobutanol/toluene (50:50) were added, and the solution was thoroughly mixed. The amount of inorganic  $\gamma$ - $^{33}\text{P}$  extracted using this method was counted with a liquid scintillation counter (Packard Instruments, Groningen, The Netherlands). Results are expressed as the percentage of tyrosine phosphatase activity in the absence of BK (time 0).

### Statistical Analysis

Data are expressed as means  $\pm$  SE of  $n$  independent experiments. Body weight, glycemia, and tyrosine phosphatase activity results were analyzed by 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 test were used for Western blot densitometric analysis. Only  $P < 0.05$  was considered significant. All analyses were performed with SigmaStat 1.0 software (Jandel).

## RESULTS

### Ex Vivo Experiments

**Effects of BK, IGF-1, PDGF-BB, VEGF, and bFGF on glomerular ERK1 and -2 phosphorylation.** Before studying the possible interaction between  $B_2R$  and growth factor receptor signaling, we studied the effect on ERK1/2 phosphorylation of separate activation of these receptors in IG. The  $B_2R$  agonist BK at 100 nM

induced a time-dependent and transient phosphorylation of ERK1/2 (p44 and p42) that peaked at 2 min, reaching 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, D and E, lanes 20–24 and 26–30, respectively). Total ERK1 expression remained unchanged during all these stimulations.

**$B_2R$  activation reduced growth factor-induced glomerular ERK1 and -2 phosphorylation.** Next, we studied the effect of a pretreatment with BK and  $B_1$ -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  $B_2R$  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  $\text{Na}^+\text{-K}^+\text{-ATPase}$  transporter by OV. However, the inhibitory effect of BK on IGF-1-induced ERK1/2 phosphorylation was not blocked by the  $\text{Na}^+\text{-K}^+\text{-ATPase}$ -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-

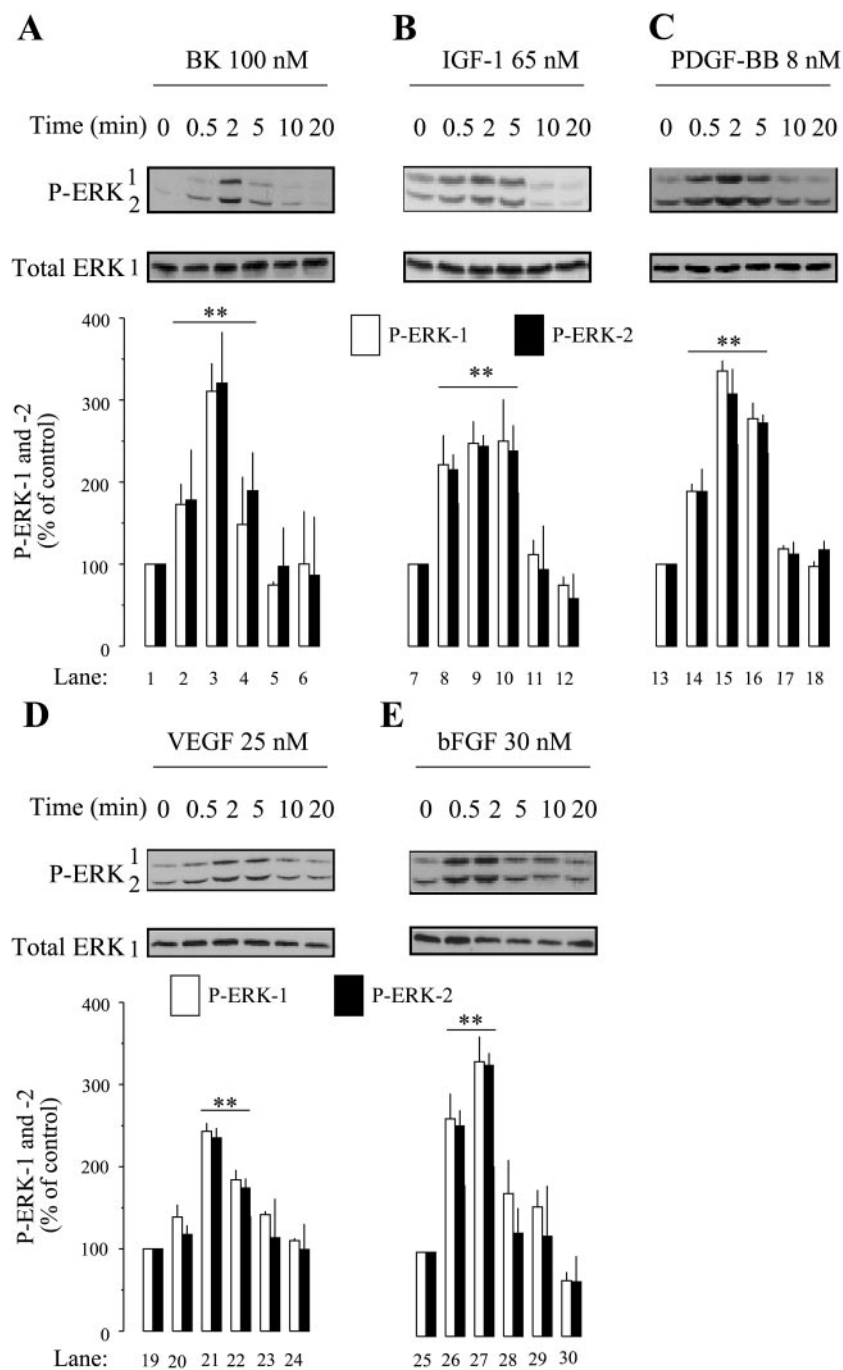


Fig. 1. Time-dependent effect of 100 nM bradykinin (BK; A), 65 nM IGF-1 (B), 8 nM PDGF-BB (C), 25 nM VEGF (D), and 30 nM basic (b)FGF (E) on ERK1/2 phosphorylation in isolated glomeruli (IG). IG were incubated in the presence of either peptide during different times (from 30 s to 20 min). ERK1/2 phosphorylation was measured by Western blotting with an antibody against the phosphorylated forms. Analysis were performed on an equal amount of protein (25  $\mu$ g) as measured with an antiserum against total ERK1 (phosphorylated and nonphosphorylated forms). The ERK1/2 phosphorylation (P-ERK) was expressed as the fold-increase of the P-ERK vs. total ERK1 ratio compared with control (time 0), and results are shown as means  $\pm$  SE of the scanning densitometry of 5 experiments.  $**P < 0.01$  compared with control level in the absence of growth factor.

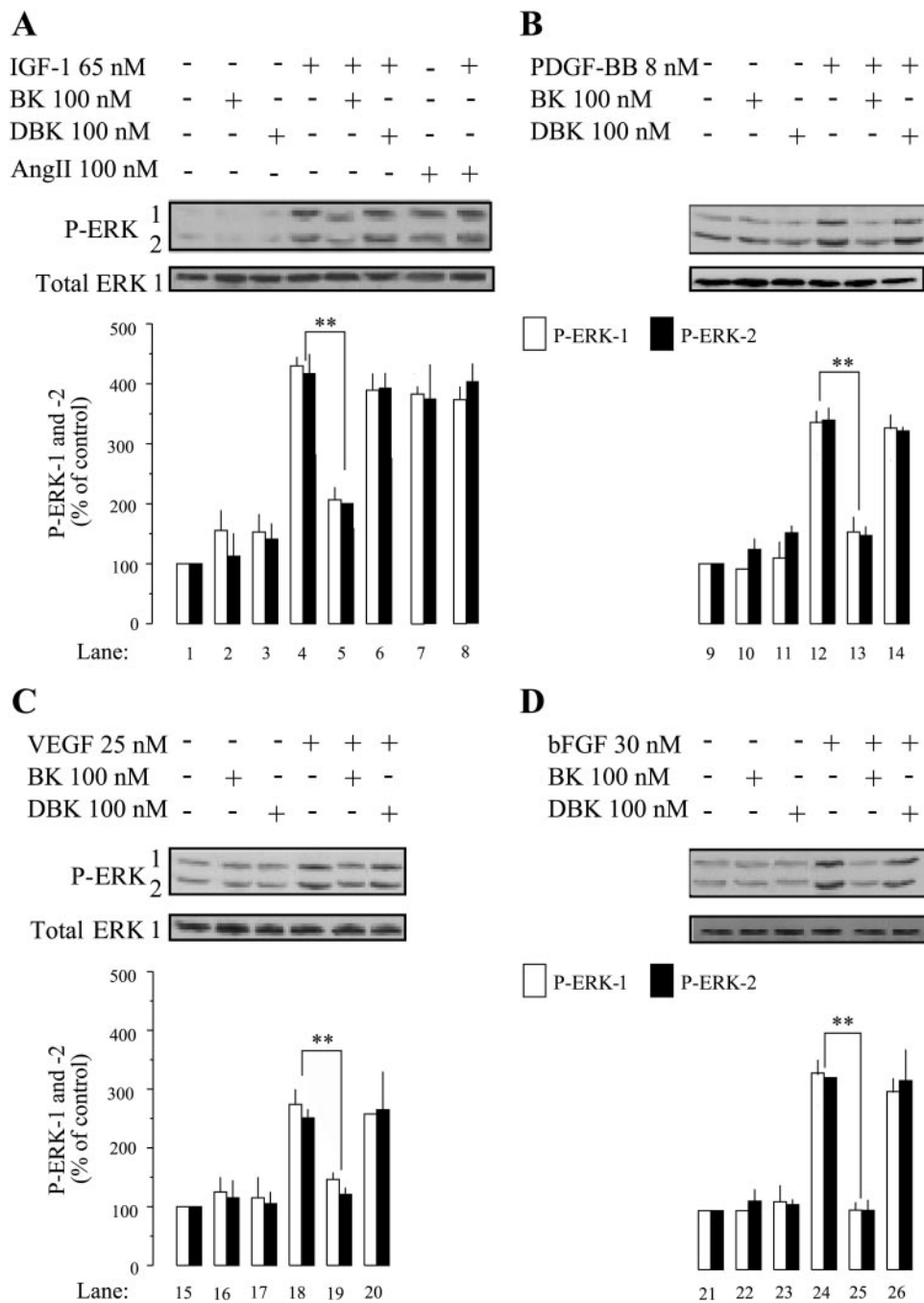
strated that BK induced a time-dependent increase in tyrosine phosphatase activity, reaching a significant 250% maximum increase after 10-min stimulation with 100 nM BK ( $P < 0.01$ ).

*MKP-1 is not involved in the negative cross talk between  $B_2R$  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  $B_2R$  and IGF-1R.*  $B_2R$  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 IG with 100  $\mu$ M L-NAME did not alter the inhibition of IGF-1-induced

Fig. 2. Effect of BK, des-Arg<sup>9</sup>-BK (DBK), and angiotensin II on IGF-1 (A)-, PDGF-BB (B)-, VEGF (C)-, and bFGF-induced (D) ERK1/2 phosphorylation in IG. IG were incubated with IGF-1, PDGF-BB, VEGF, or bFGF for 2 min, whereas BK, DBK, and angiotensin II were added 18 min beforehand. Western blot analysis was performed as described in the legend of Fig. 1. \*\**P* < 0.01 compared with the maximum effect of growth factor alone (*n* = 5).



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 B<sub>2</sub>R 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.

*B<sub>2</sub>R 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 B<sub>2</sub>R with HOE-140 (lane 5 vs. 4; *P* < 0.01).

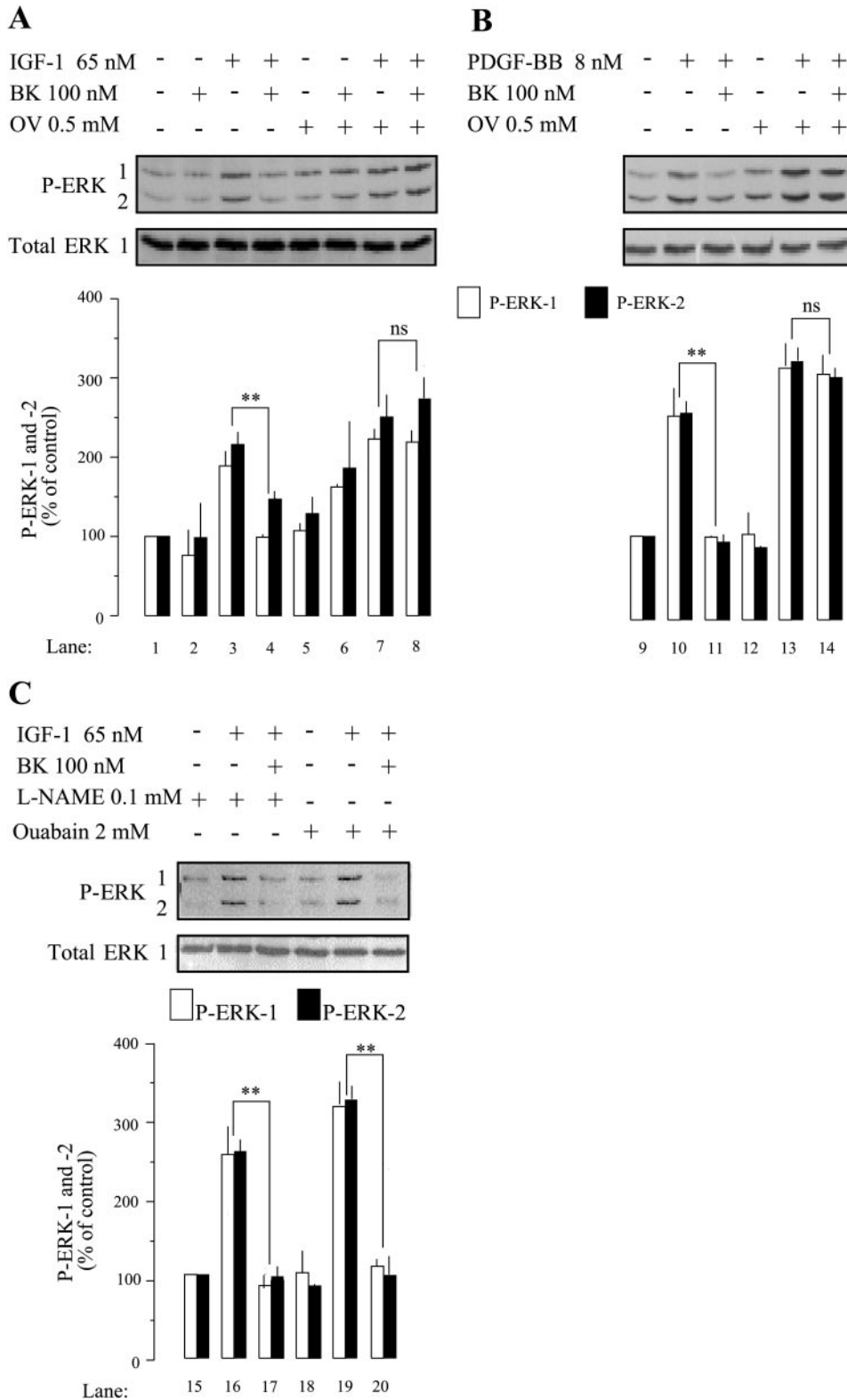


Fig. 3. Blockade of the inhibitory effect of BK on IGF-1 (A)- and PDGF-BB (B)-induced ERK1/2 phosphorylation in IG with the tyrosine phosphatase inhibitor orthovanadate (OV) or with either  $N^G$ -nitro-L-arginine methyl ester (L-NAME) or ouabain (C). IG were incubated with IGF-1 or PDGF-BB for 2 min, whereas BK (18 min), OV (50 min), L-NAME (50 min), and ouabain (20 min) were added beforehand. Western blot analysis was performed as described in the legend of Fig. 1. ns, Not significant.  $**P < 0.01$  compared with the maximum effect of IGF-1 or PDGF-BB alone ( $n = 5$ ).

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.

*B<sub>2</sub>R* 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

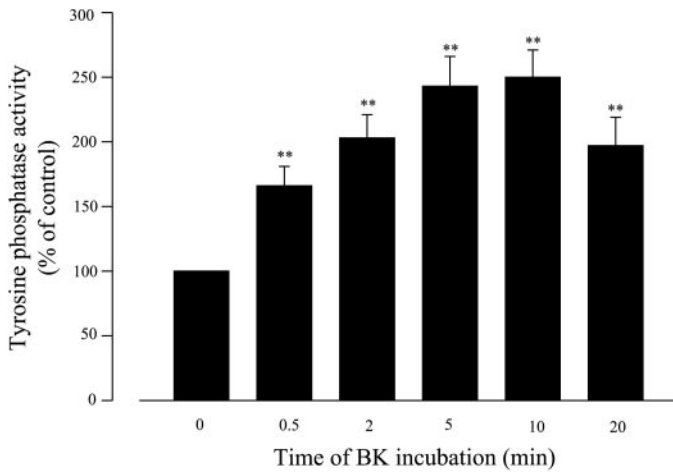


Fig. 4. BK-induced increase in total tyrosine phosphatase activity. IG were incubated with 100 nM BK for the time indicated. Total tyrosine phosphatase activity was determined using <sup>33</sup>P-labeled poly(Glu-Tyr). Data are expressed as the percentage of free <sup>33</sup>P observed in nontreated IG (time 0). Basal total tyrosine phosphatase activity in nontreated IG was 52 cpm·min<sup>-1</sup>·10 μg protein<sup>-1</sup>, where cpm is counts/min. Results are expressed as means ± SE of 3 independent experiments. \*\*P < 0.01 compared with control (time 0).

(P < 0.01; n = 4). 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).

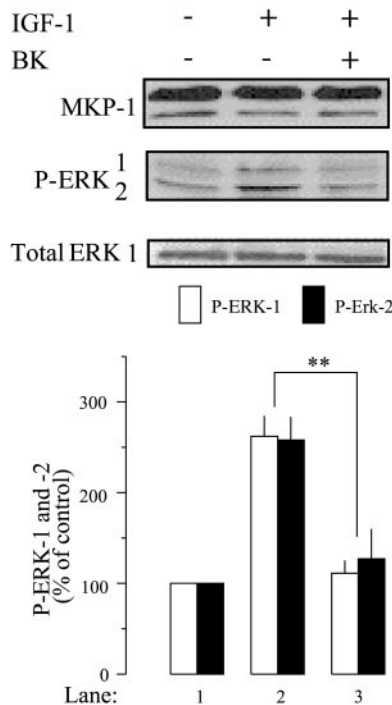


Fig. 5. Effect of IGF-1 and BK on MAPK phosphatase-1 (MKP-1) protein expression and ERK1/2 phosphorylation in IG. IG were incubated with IGF-1 for 2 min, whereas BK was added 18 min beforehand. Western blot analysis was performed as described in the legend of Fig. 1, with antibodies directed against MKP-1 and P-ERK1/2 (n = 6). \*\*P < 0.01 compared with IGF-1 without BK (lanes 2 and 3).

Table 1. Data for rats included in in vivo experiments

Treatment	Wt		Glycemia After Treatment, mg/dl
	Before treatment, g	After treatment, g	
Control	370 ± 2	374 ± 3	93 ± 9
STZ	365 ± 9	308 ± 7*	336 ± 13†
STZ+insulin	369 ± 12	379 ± 18	108 ± 16
STZ+ramipril	351 ± 7	306 ± 20*	335 ± 41†
STZ+ramipril+HOE-140	343 ± 7	285 ± 5*	399 ± 12†
Losartan	347 ± 13	297 ± 8*	354 ± 23†

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 B<sub>2</sub>R with HOE-140 (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 B<sub>2</sub>R activation and involves tyrosine phos-

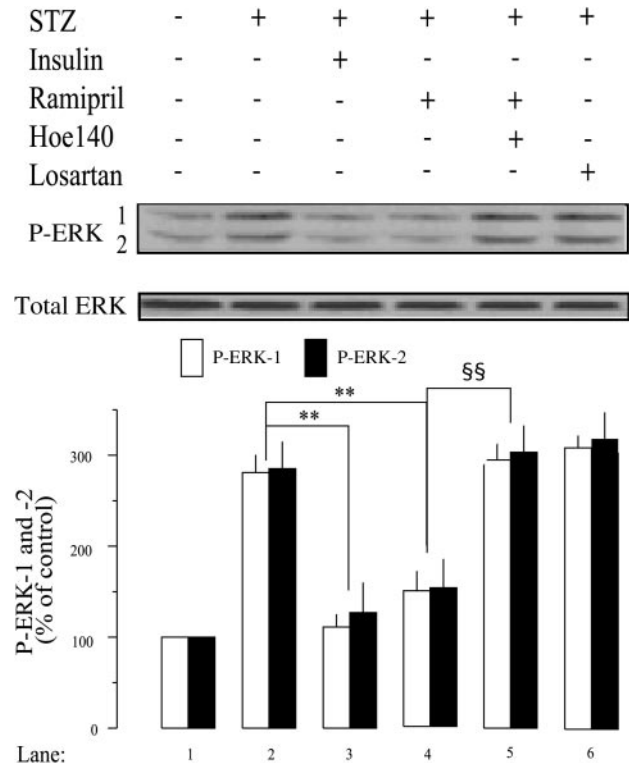


Fig. 6. Effect of B<sub>2</sub> receptor (B<sub>2</sub>R) blockade on ERK1/2 phosphorylation in IG of streptozotocin (STZ)-diabetic rats. STZ-diabetic rats were either untreated or received insulin (2 U/day), an angiotensin-converting enzyme (ACE) inhibitor (ramipril; 1 mg·kg<sup>-1</sup>·day<sup>-1</sup>) and/or the B<sub>2</sub>R antagonist HOE-140 (0.25 mg·kg<sup>-1</sup>·day<sup>-1</sup>) and an angiotensin AT<sub>1</sub> receptor blocker (losartan; 10 mg·kg<sup>-1</sup>·day<sup>-1</sup>). Western blot analysis was performed as described in the legend of Fig. 1 (n = 4). \*\*P < 0.01 compared with diabetic rats (lane 2). §§P < 0.01 compared with diabetic rats treated with ramipril.

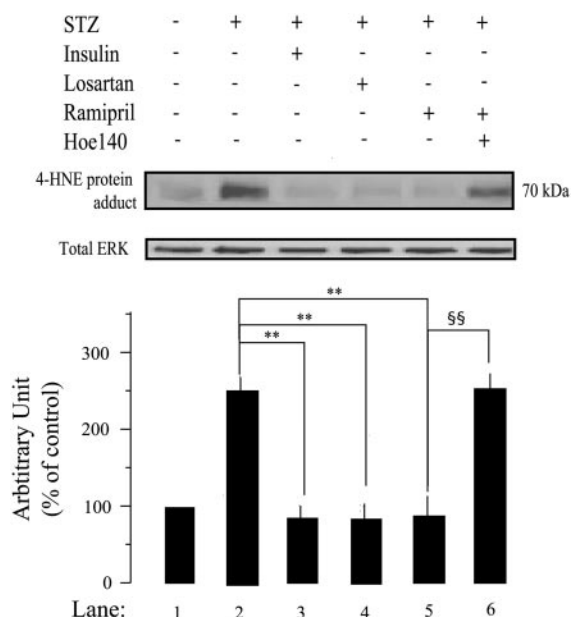


Fig. 7. Effect of B<sub>2</sub>R blockade on 4-hydroxynonenal (4-HNE) protein derivatives labeling in IG of STZ-diabetic rats. STZ-diabetic rats were either untreated or received insulin (2 U/day), losartan (10 mg·kg<sup>-1</sup>·day<sup>-1</sup>), ramipril (1 mg·kg<sup>-1</sup>·day<sup>-1</sup>), and/or HOE-140 (0.25 mg·kg<sup>-1</sup>·day<sup>-1</sup>). 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 B<sub>2</sub>R 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 B<sub>2</sub>R 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 B<sub>2</sub>R 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 B<sub>2</sub>R 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 B<sub>2</sub>R activation of IGF-1, PDGF-BB, VEGF, and bFGF signaling might be of physiological 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- $\beta$  and to mediate TGF- $\beta$ -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 therapeutical 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 B<sub>2</sub>R 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 B<sub>2</sub>R activation, because blockade of the B<sub>2</sub>R 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 B<sub>2</sub>R (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-I-induced ERK1/2 phosphorylation. Although both ACE inhibitors and AT<sub>1</sub> 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 B<sub>2</sub>R 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 B<sub>2</sub>R, 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 B<sub>2</sub>R 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 B<sub>2</sub>R 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 B<sub>2</sub>R 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.

The authors acknowledge Aventis Pharma (Germany) for providing ramipril and HOE-140 and Merck (US) for losartan. The authors also acknowledge the technical assistance of Denis Calise in managing the insulin implants as well as Dr. Joost P. Schanstra for helpful suggestions during the writing of the manuscript.

E. Cellier is supported by a Fellowship from the Fonds de la Recherche en Santé du Québec.

## REFERENCES

1. **Abboud HE.** Growth factors in glomerulonephritis. *Kidney Int* 43: 252–267, 1993.
2. **Alric C, Pecher C, Bascands JL, and Girolami JP.** Effect of bradykinin on tyrosine kinase and phosphatase activities and cell proliferation in mesangial cells. *Immunopharmacology* 45: 57–62, 1999.
3. **Amemiya T, Sasamura H, Mifune M, Kitamura Y, Hirahashi J, Hayashi M, and Saruta T.** Vascular endothelial growth factor activates MAP kinase and enhances collagen synthesis in human mesangial cells. *Kidney Int* 56: 2055–2063, 1999.
4. **Asada T, Ogawa T, Iwai M, Shimomura K, and Kobayashi M.** Recombinant insulin-like growth factor I normalizes expression of renal glucose transporters in diabetic rats. *Am J Physiol Renal Physiol* 273: F27–F37, 1997.
5. **Awazu M, Ishikura K, Hida M, and Hoshiya M.** Mechanisms of mitogen-activated protein kinase activation in experimental diabetes. *J Am Soc Nephrol* 10: 738–745, 1999.
6. **Bascands JL, Pecher C, Rouaud S, Emond C, Tack Y, Bastie MJ, Burch R, Regoli D, and Girolami JP.** Evidence for existence of two distinct bradykinin receptors on rat mesangial cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F548–F556, 1993.
7. **Beltman J, McCormick F, and Cook SJ.** The selective protein kinase C inhibitor, Ro-31–8220, inhibits mitogen-activated protein kinase phosphatase-1 (MKP-1) expression, induces c-Jun expression, and activates Jun N-terminal kinase. *J Biol Chem* 271: 27018–27024, 1996.
8. **Blazer-Yost BL, Goldfarb S, and Ziyadeh FN.** Insulin, insulin-like growth factors, and the kidney. In: *Hormones, Autacoids, and the Kidney*, edited by Goldfarb S, Ziyadeh FN, and Stein JH. New York: Churchill Livingstone, 1991, p. 339–363.
9. **Bokemeyer D, Ostendorf T, Kunter U, Lindemann M, Kramer HJ, and Floege J.** Differential activation of mitogen-activated protein kinases in experimental mesangioproliferative glomerulonephritis. *J Am Soc Nephrol* 11: 232–240, 2000.
10. **Campbell DJ, Kladis A, and Duncan AM.** Bradykinin peptides in kidney, blood, and other tissues of the rat. *Hypertension* 21: 155–165, 1993.
11. **Cha DR, Kim NH, Yoon JW, Jo SK, Cho WY, Kim HK, and Won NH.** Role of vascular endothelial growth factor in diabetic nephropathy. *Kidney Int* 58: S104–S112, 2000.
12. **Chin TY, Lin YS, and Chueh SH.** Antiproliferative effect of nitric oxide on rat glomerular mesangial cells via inhibition of mitogen-activated protein kinase. *Eur J Biochem* 268: 6358–6368, 2001.
13. **Choudhury GG, Karamitsos C, Hernandez J, Gentilini A, Bardgette J, and Abboud HE.** PI-3-kinase and MAPK regulate mesangial cell proliferation and migration in response to PDGF. *Am J Physiol Renal Physiol* 273: F931–F938, 1997.
14. **Coolican SA, Samuel DS, Ewton DZ, McWade FJ, and Florini JR.** The mitogenic and myogenic actions of insulin-like growth factors utilize distinct signaling pathways. *J Biol Chem* 272: 6653–6662, 1997.
15. **Dixon BS and Dennis MJ.** Regulation of mitogenesis by kinins in arterial smooth muscle cells. *Am J Physiol Cell Physiol* 273: C7–C20, 1997.
16. **Doi T, Striker LJ, Elliot SJ, Conti FG, and Striker GE.** Insulin-like growth factor-1 is a progression factor for human mesangial cells. *Am J Pathol* 134: 395–404, 1989.
17. **Doi T, Stricker LJ, Gibson CC, Agodoa LYC, Brinster RL, and Stricker GE.** Glomerular lesions in mice transgenic for growth hormone and insulin like growth factor-I. *Am J Pathol* 137: 541–552, 1990.
18. **Douillet CD, Velarde V, Christopher JT, Mayfield RK, Trojanowska ME, and Jaffa AA.** Mechanisms by which bradykinin promotes fibrosis in vascular smooth muscle cells: role of TGF- $\beta$  and MAPK. *Am J Physiol Heart Circ Physiol* 279: H2829–H2837, 2000.
19. **Duchêne J, Schanstra JP, Pécher C, Pizard A, Susini C, Esteve JP, Bascands JL, and Girolami JP.** A novel protein-protein interaction between a G-protein coupled receptor and phosphatase SHP-2 is involved in bradykinin-induced inhibition of cell proliferation. *J Biol Chem* 277: 40375–40383, 2002.
20. **El-Dahr SS, Dipp S, and Baricos WH.** Bradykinin stimulates the Erk-Elk-1-Fos/AP-1 pathway in mesangial cells. *Am J Physiol Renal Physiol* 275: F343–F352, 1998.
21. **Feld SM, Hirschberg R, Artishevsky A, Nast C, and Adler SG.** Insulin-like growth I induces mesangial proliferation and increases mRNA and secretion of collagen. *Kidney Int* 48: 45–51, 1995.
22. **Fenoy FJ, Scicli G, Carretero O, and Roman RJ.** Effect of an angiotensin II and a kinin receptor antagonist on the renal hemodynamic response to captopril. *Hypertension* 17: 1038–1044, 1991.
23. **Flyvberg A.** Putative pathophysiological role of growth factors and cytokines in experimental diabetic kidney disease. *Diabetologia* 43: 1205–1233, 2000.
24. **Gilbert RE, Cox A, Wu LL, Allen TJ, Hulthen UL, Jerums G, and Cooper ME.** Expression of transforming growth factor- $\beta$ 1 and type IV collagen in the renal tubulointerstitium in experimental diabetes. Effects of ACE inhibition. *Diabetes* 47: 414–422, 1998.
25. **Graness A, Adomeit A, Heinze R, Wetzker R, and Liebmann C.** A novel mitogenic signaling pathway of bradykinin in the human colon carcinoma cell line SW-480 involves sequential activation of a G $_{q/11}$  protein, phosphatidylinositol 3-kinase  $\beta$ , and protein kinase C $\epsilon$ . *J Biol Chem* 273: 32016–32022, 1998.
26. **Graness A, Hanke S, Boehmer FD, Presek P, and Liebmann C.** Protein-tyrosine-phosphatase-mediated transactivation and EGF receptor-independent stimulation of mitogen-activated protein kinase by bradykinin in A431 cells. *Biochem J* 347: 441–447, 2000.
27. **Ha H and Kim KH.** Pathogenesis of diabetic nephropathy: the role of oxidative stress and protein kinase C. *Diabetes Res Clin Pract* 45: 147–151, 1999.
28. **Ha H and Lee HB.** Reactive oxygen species as glucose signaling molecules in mesangial cells cultured under high glucose. *Kidney Int* 58: S19–S25, 2000.
29. **Hajinazarian M, Cosio FG, Nahman NS, and Mahan JD.** Angiotensin-converting enzyme inhibition partially prevents diabetic organomegaly. *Am J Kidney Dis* 23: 105–117, 1994.
30. **Hamaguchi A, Kim S, Izumi Y, and Iwao H.** Chronic activation of glomerular mitogen-activated protein kinases in Dahl salt-sensitive rats. *J Am Soc Nephrol* 11: 39–46, 2000.
31. **Haneda M, Araki S, Togawa M, Sugimoto T, Isono M, and Kikkawa R.** Mitogen-activated protein kinase cascade is activated in glomeruli of diabetic rats and glomerular mesangial cells cultured under high glucose conditions. *Diabetes* 46: 847–853, 1997.
32. **Hänsch GM, Wagner C, Bürger A, Dong W, Staehler G, and Stoeck M.** Matrix protein synthesis by glomerular mesangial cells in culture: effects of transforming growth factor  $\beta$  (TGF $\beta$ ) and platelet-derived growth factor (PDGF) on fibronectin and collagen type IV mRNA. *J Cell Physiol* 163: 451–457, 1995.
33. **Hayashida T, Poncelet AC, Hubchak SC, and Schnaper HW.** TGF- $\beta$ 1 activates MAP kinase in human mesangial cells: a possible role in collagen expression. *Kidney Int* 56: 1710–1720, 1999.
34. **Heilig CW, Liu Y, England RL, Freytag SO, Gilbert JD, Heilig KO, Zhu M, Concepcion LA, and Brosius FC III.** D-Glucose stimulates mesangial cell GLUT1 expression and basal and IGF-1-sensitive glucose uptake in rat mesangial cells: implications for diabetic nephropathy. *Diabetes* 45: 1030–1039, 1997.
- 34a. **Huang W, Gallois Y, Bouby N, Bruneval P, Heudes D, Belair MF, Krege JH, Meneton P, Marre M, Smithies O, and Alhenc-Gelas F.** Genetically increased angiotensin I-converting enzyme level and renal complications in the diabetic mouse. *Proc Natl Acad Sci USA* 98: 13330–13334, 2001.
35. **Isono M, Iglesias-De La Cruz MC, Chen S, Hong SW, and Ziyadeh SN.** Extracellular signal-regulated kinase mediates stimulation of TGF- $\beta$ 1 and matrix by high glucose in mesangial cells. *J Am Soc Nephrol* 11: 2222–2230, 2000.
36. **Johnson R, Ida H, Yoshimura A, Floege J, and Bowen-Pope DF.** Platelet-derived growth factor: a potentially impor-

- tant cytokine in glomerular disease. *Kidney Int* 41: 590–594, 1992.
37. **Keane F.** Metabolic pathogenesis of cardiovascular disease. *Am J Kidney Dis* 38: 1372–1375, 2001.
  38. **Lewis EJ, Hunsicker LG, Bain RP, and Rohde RD.** The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N Engl J Med* 329: 1456–1462, 1993.
  39. **Mattson DL and Roman RJ.** Role of kinins and angiotensin in the renal hemodynamic response to captopril. *Am J Physiol Renal Fluid Electrolyte Physiol* 260: F670–F679, 1991.
  40. **Mukai H, Fitzgibbon WR, Ploth DW, and Margolius HS.** Effect of chronic bradykinin B<sub>2</sub> receptor blockade on blood pressure of conscious Dahl salt-resistant rats. *Br J Pharmacol* 124: 197–205, 1998.
  41. **Nakamura T, Fukui M, Ebihara I, Osada S, and Nagaoka I.** mRNA expression of growth factors in glomeruli from diabetic rats. *Diabetes* 42: 450–456, 1993.
  42. **Olcese L, Lang P, Vely F, Cambiaggi A, Marguet D, Blery M, Hippen KL, Biassoni R, Moretta A, Moretta L, Cambier JC, and Vivier E.** Human and mouse killer-cell inhibitory receptors recruit PTP1C and PTP1D protein tyrosine phosphatases. *J Immunol* 156: 4531–4534, 1996.
  43. **Patel KI and Schrey MP.** Inhibition of DNA synthesis and growth in human breast stromal cells by bradykinin: evidence for independent roles of B<sub>1</sub> and B<sub>2</sub> receptors in the respective control of cell growth and phospholipid hydrolysis. *Cancer Res* 52: 334–340, 1992.
  44. **Shankland SJ, Pippin J, Flanagan M, Coats SR, Nangaku M, Gordon KL, Roberts JM, Couser WG, and Johnson RJ.** Mesangial cell proliferation mediated by PDGF and bFGF is determined by levels of the cyclin kinase inhibitor p27<sup>Kip1</sup>. *Kidney Int* 51: 1088–1099, 1997.
  45. **Smithies O, Kim HS, Takahashi N, and Edgell MH.** Importance of quantitative genetic variations in the etiology of hypertension. *Kidney Int* 58: 2265–2280, 2000.
  46. **Strutz F, Zeisberg M, Hemmerlein B, Sattler B, Hummel K, Becker V, and Müller GA.** Basic fibroblast growth factor expression is increased in human renal fibrogenesis and may mediate autocrine fibroblast proliferation. *Kidney Int* 57: 1521–1538, 2000.
  47. **Suzuki D, Miyata T, Saotome N, Horie K, Inagi R, Yasuda Y, Uchida K, Izuhara Y, Yagame M, Sakai H, and Kurokawa K.** Immunohistochemical evidence for an increased oxidative stress and carbonyl modification of proteins in diabetic glomerular lesions. *J Am Soc Nephrol* 10: 822–832, 1999.
  48. **Tschöpe C, Reinecke A, Seidl U, Yu M, Gavrilluk V, Riester U, Gohlke P, Graf K, Bader M, Hilgenfeldt U, Pesquero JB, Ritz E, and Unger T.** Functional, biochemical, and molecular investigation of renal kallikrein-kinin system in diabetic rats. *Am J Physiol Heart Circ Physiol* 277: H2333–H2340, 1999.
  49. **Tsuchida S, Miyazaki Y, Matsusaka T, Hunley TE, Inagami T, Fogo A, and Ichakawa I.** Potent antihypertrophic effect of the bradykinin B<sub>2</sub> receptor system on the renal vasculature. *Kidney Int* 56: 509–516, 1999.
  50. **Velarde V, Ullian ME, Morinelli TA, Mayfield RK, and Jaffa AA.** Mechanism of MAPK activation by bradykinin in vascular smooth muscle cells. *Am J Physiol Cell Physiol* 277: C253–C261, 1999.
  51. **Wenzel UO, Fouqueray B, Biswas P, Grandaliano G, Choudhury GG, Abboud HE.** Activation of mesangial cells by the phosphatase inhibitor vanadate. *J Clin Invest* 95: 1244–1252, 1995.
  52. **Werner H, Shen-Orr Z, Stannard B, Burguera B, Roberts CT, and Leroith D.** Experimental diabetes increases insulin like growth factor I and II receptor concentration and gene expression in kidney. *Diabetes* 39: 1490–1497, 1990.
  53. **Wolf G and Ziyadeh FN.** Molecular mechanisms of diabetic renal hypertrophy. *Kidney Int* 56: 393–405, 1999.
  54. **Yavuz DG, Ersoz O, Kucukkaya B, Budak Y, Ahiskali R, Ekicioglu G, Emerk K, and Akalin S.** The effect of losartan and captopril on glomerular basement membrane anionic charge in a diabetic rat model. *J Hypertens* 17: 1217–1223, 1999.
  55. **Young BA, Johnson RJ, Alpers CA, Eng E, Gordon K, Floege J, and Couser WG.** Cellular events in the evolution of experimental diabetic nephropathy. *Kidney Int* 47: 935–944, 1995.