Insulin increases the activity of mesangial BK channels through MAPK signaling

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Foutz RM, Grimm PR, Sansom SC. Insulin increases the activity of mesangial BK channels through MAPK signaling. Am J Physiol Renal Physiol 294: F1465–F1472, 2008. First published March 26, 2008; doi:10.1152/ajprenal.00012.2008.—Glomerular hyperfiltration and mesangial expansion have been described in mouse models of a hyperinsulinemic early stage of type 2 diabetes mellitus (DM). Large-conductance Ca\(^{2+}\)-activated K\(^{+}\) channels (BK) have been linked to relaxation of human mesangial cells (MC) and may contribute to MC expansion and hyperfiltration. We hypothesized that high insulin levels increase BK activity in MC by increasing the number and/or open probability (\(P_o\)) of BK in the plasma membrane. With the use of the patch-clamp technique, BK activity was analyzed in cultured MC exposed to normal insulin (1 nM) and high insulin (100 nM) for a 48-h period. The mean \(P_o\) and the percentage of patches (cell attached) with detected BK increased by 100% in the insulin-treated cells. Real-time PCR revealed that insulin increased mRNA of BK-\(\alpha\). Western blot revealed an insulin-stimulated increase in BK-\(\alpha\) from both total cellular and plasma membrane protein fractions. The mitogen-activated protein kinase (MAPK) inhibitors PD-098059 and U-0126 attenuated the insulin-induced increase in BK-\(\alpha\) expression. PD-098059 inhibited insulin-stimulated phosphorylation of extracellular signal-regulated kinase 1/2 in MC. An insulin-stimulated increase was found for total cellular BK-\(\beta_1\), the accessory subunit of BK in MC. A similar increase in BK-\(\alpha\) mRNA and protein was evoked by an insulin-like growth factor I receptor (13) analog. Glomeruli, isolated from hyperinsulinemic early stage type 2 DM mice, exhibited increased BK-\(\alpha\) mRNA by real-time PCR and protein by immunohistochemical staining and Western blot. These results indicate that insulin activates BK in the plasma membrane of MC and stimulates, via MAPK, an increase in cellular and plasma membrane BK-\(\alpha\).

insulin-like growth factor I receptor; maxi k; potassium channel; \(\beta_1\)-subunit; mouse; high-fat diet

glomerular filtration rate (14, 43). In MC, BK are composed of pore-forming \(\alpha\) and accessory \(\beta_1\)-subunits (24). The \(\beta_1\)-subunits increase Ca\(^{2+}\) and voltage sensitivity of BK (7, 29).

Previously, we described hyperfiltration and mesangial expansion in a high-fat diet mouse model of early stage type 2 diabetes mellitus (DM) (45). Although mildly hyperglycemic, the DM mice exhibited profound hyperinsulinemia. Considering the functional role that BK have in MC, we hypothesized that high plasma insulin levels increase the activity of BK by either increasing their open probability (\(P_o\)) or protein expression in the plasma membrane. The increased activity of BK could account for the relaxation of MC, providing a possible mechanism for the hyperfiltration in the type 2 DM model.

MC possess both insulin receptors (IR) and insulin-like growth factor I receptors (IGF-IR) (13). Both are tyrosine kinase receptors that share amino acid sequence homology and mutual intracellular signaling pathways (2). The amount of IR in MC is low compared with IGF-IR (13); however, elevated levels of insulin are sufficient to activate IGF-IR (2, 4) and stimulate mesangial proliferation (22). Insulin-like growth factor I (IGF-I) is elevated in glomeruli of type 2 DM models, and infusion of IGF-I increases glomerular filtration rate in rats (19).

Activating BK may also be partly responsible for the mesangial expansion noted in the hyperinsulinemic stage of type 2 DM (11, 45). Activation of BK hyperpolarizes the membrane potential and results in an enhanced driving force for Ca\(^{2+}\) cell entry. Activation of BK increases proliferation of breast cancer cells in vitro (12). Moreover, Ca\(^{2+}\) channel blockers inhibit MC proliferation (36).

A recent study described activation of BK in hippocampal cells via the mitogen-activated protein kinase (MAPK) pathway (31), which is stimulated via IGF-IR in many types of cells (39). We therefore explored whether insulin evoked an increase in mesangial BK activity, possibly by stimulating the MAPK pathway.

METHODS

Cell culture. Human MC (Cambrex, East Rutherford, NJ), passages 6–11, were grown in 5 mM glucose DMEM (Sigma, St. Louis, MO) supplemented with 10 mM HEPES, 2.0 mM glutamine, 1.0 mM sodium pyruvate, 0.1 mM nonessential amino acids, 100 U/ml penicillin, 100 \(\mu\)g/ml streptomycin, and 20% FBS. In a blind study, human MC were either further supplemented with 100 nM human insulin (Sigma) or 5 nM IGF-I analog (Novozymes Gropep) in 2% FBS, to mimic hyperinsulinemia, or incubated with standard 1 nM insulin DMEM with 20% FBS over a 48-h period. In separate experiments, cells were preincubated with MAPK inhibitors, 10 \(\mu\)M PD-098059.
Pittsburgh, PA) for patch-clamp experiments and in 75-cm² flasks of BK in cell-attached patches (Mc). Cells were cultured on 22 × 22 mm glass cover slips (Fisher, Pittsburgh, PA) for patch-clamp experiments and in 75-cm² flasks of BK in cell-attached patches (Mc).

**Patch-clamp experiments.** Single-channel patch-clamp experiments were performed at 23°C as previously described (18). Experiments began in the cell-attached configuration. Seals maintained throughout the experiment were eventually excised to the inside-out configuration to confirm the number of BK in the patch. The pipette solution was (in mM): 140 KCl, 5 KCl, 2 MgCl₂, 1 CaCl₂, and 10 HEPES (pH 7.4). The bath solution contained standard physiological saline solution [(in mM) 135 NaCl, 5 KCl, 2 MgCl₂, 1 CaCl₂, and 10 HEPES, pH 7.4].

Pipettes were manufactured to a 3-mm taper and a 1-µm tip diameter using a model P-97 horizontal pipette puller (Sutter Instrument, Novato, CA). The patch pipette, filled with solution and in contact with aAg-AgCl wire, was connected to a model CV 203BU head stage attached to an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). The cells, grown on cover slips, were mounted an imaging platform and visualized with an Olympus (OMT2) inverted microscope. The pipette was lowered on the cell membrane. Once contact was established, suction was applied to form a high-resistance (>5 GΩ) seal. Multiple current tracings were recorded at 20-mV increments to establish BK identification, activity, and single-channel conductance.

Single-channel events were acquired with pCLAMP 10.0 software (Molecular Devices) and analyzed using Clampfit software (Molecular Devices). The Pₖ, percentage of time a channel remains in the open state divided by the total time of the analyzed record, was calculated for each BK observed in a patch. The channel activity was initially calculated as NPₙ = EnPₙ, where Pₙ is the probability of finding n channels open. The N was determined by observing the maximum number of current levels in the patch after excision in the bathing solution containing 1 mM Ca²⁺. Therefore, the Pₙ was determined by dividing the NPₙ by N. Only seals containing distinguishable BK that could be identified by a current-voltage (I-V) plot were evaluated.

**Protein isolation and Western blotting.** Standard cellular protein isolation and Western blot procedure was performed. The isolated protein concentration was determined with a standard curve using Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA). To isolate membrane protein, a 1-h centrifugation at 100,000 g was performed as previously described (40). The supernatant (cytosolic protein) was collected, and the pellet was resuspended in RIP buffer, briefly sonicated, and centrifuged. The supernatant (membrane protein) was collected and analyzed. PhosphoSafe Extraction Buffer (Novagen, Gibbstown, NJ) was used to isolate protein from human MC to detect phosphorylated proteins. All isolated protein was stored at −80°C until analyzed.

Western blotting was performed as previously described (42). The primary antibodies included anti-BK-α (rabbit; APC-107 and APC-021; Alomone), anti-BK-β (goat; Santa Cruz Biotechnology, Santa Cruz, CA), anti-β-actin (rabbit; Santa Cruz), anti-caveolin-1 (rabbit; Affinity Bioreagents, Golden, CO), anti-phosphorylated extracellular signal-regulated kinase (ERK) 1/2 (rabbit; no. 9101; Cell Signaling Technology, Danvers, MA), or ERK1/2 (rabbit; no. 9102; Cell Signaling Technology) diluted 1:1,000 in Tris-buffered saline supplemented with 0.05% Tween 20 (TBST) and 3% BSA. The following day the blot was washed and incubated with a horseradish peroxidase (HRP) conjugated secondary antibody diluted 1:5,000 in TBST and 1% BSA. The blot was washed and the HRP labeling was developed with SuperSignal West Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL). The blots were analyzed using UVP Imager and Labworks software.

**RNA isolation and real-time PCR.** RNA was isolated from human MC with TRI REAGENT (Molecular Research Center, Cincinnati, OH) per the manufacturer’s protocol. The isolated RNA was reverse-transcribed with Superscript III (Invitrogen, Carlsbad, CA) and oligo(dT) primers using the manufacturer’s protocol. All real-time PCR experiments were performed with iQ SYBR Green Supermix (Bio-Rad). Each reaction mix consisted of 12.5 µl iQ SYBR Green Supermix, 1 µl of 10 mM sense primer, 1 µl of 10 mM anti-sense primer, 0.5 µl of cDNA template, and water for a total volume of 25 µl. The nucleotide sequence for BK-α is sense AAGGGCTGTCA-ACATCAACC, antisense GATGTGTAGTGACGCCAAGA; for gliceraldehyde-3-phosphate dehydrogenase (GAPDH), sense AA-CAGCCTCAAGATCATCAGC, antisense GGATGATGTCCTG-GACGCCAAGA-

**Fig. 1. Effects of insulin on large-conduc-
tance Ca²⁺-activated K⁺ channels (BK) in cell-attached patches of cultured human mes-
angial cells (MC). A: representative tracings of BK in cell-attached patches (−Vₑ = −40 mV) from control (top) and insulin-treated (bottom) human MC. B: bar plot illustrating the summary of open probabilities (Pₒ) for BK in control (n = 7) and insulin-treated (n = 7) cells. C: bar plot illustrating the percentage of cell-attached patches with detectable BK in control and insulin-treated cells. *Significance at P < 0.05 using t-test for unpaired data. D: current-voltage (I-V) relations for BK detected in cell-attached patches from control and insulin-treated cells (n = 4–7). The slope and reversal potential for BK in control cells was 159 pS and 47.0 mV, respectively.
GAGAGCC. All reaction volumes were adjusted to run each sample in triplicate. The relative expression of BK-α in control and insulin-treated cells was determined using the equation of Pfaffl et al. (33).

In vivo experiments. Male C57Bl/6 mice (Jackson Laboratory, Bar Harbor, ME) were supported in the animal facility of the University of Nebraska Medical Center. All experiments were performed under conditions reviewed and approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center.

Animals (9 wk old) were placed on a normal diet (7012; Harlan Teklad) or high-fat diet (45% kcal fat; Research Diet, New Brunswick, NJ), given water ad libitum, and exposed to a 12:12-h day-night rhythm for a period of 13 wk. The animals were weighed each week. At the end of the 13-wk period, the animals were injected with anesthetic (Inactin; Sigma), a blood sample was taken from the saphenous vein, and a urine sample was collected. The blood sample was spun [12,000 revolutions/min (rpm), 10 min, 4°C] to separate plasma from the red blood cells, and the plasma was transferred to a clean Eppendorf tube. All samples were stored at −80°C for later analysis.

Glomeruli were isolated from the left kidney as previously described (9). After a 1-h centrifugation at 8,000 rpm at 4°C, the supernatant was aspirated, and the glomeruli (pellet at the bottom) were resuspended in protease-inhibiting RIPA buffer. To isolate glomerular protein, the glomerular tissue was sonicated and centrifuged at 12,500 rpm. The supernatant (glomerular protein) was collected and stored at −80°C until further analysis.

RNA was isolated from the cortical tissue via TRI REAGENT, reverse transcribed, and analyzed by real-time PCR (described above). The mouse nucleotide sequences used for the real-time PCR experiments were BK-α sense GACGTCTGAAGCGTGACTG and antisense TGGTTGAGCAATCTTACAGAG and GAPDH sense AGGTCGGTTGAAAGGATTG and antisense TGAGACCATTGTAGTTAGGTCA.

Plasma insulin concentration was determined using an Ultra Sensitive Rat Insulin ELISA kit (Crystal Chem, Downers Grove, IL). The manufacturer’s protocol was precisely followed. Each unknown sample was determined using the standard curve.

Statistical analysis. Expression of BK-α and BK-β1 proteins was quantified by densitometry measured from the Western blots using Labworks software. The mean value for both proteins was normalized by dividing each by the mean densitometry values of β-actin for total cell protein or caveolin-1 for membrane protein. Phosphorylated-ERK1/2 expression was similarly normalized to ERK1/2. The mean and SE of multiple samples were calculated and compared with the control values using a two-tailed t-test (SigmaStat 3.0). Differences were considered statistically significant at values with P < 0.05.

The mean P_o values at pipette potentials that showed clear channel activity in control cells were compared with those treated with insulin using a Mann-Whitney Rank Sum Test (SigmaStat 3.0). The appearance of BK openings was inconsistent at different potentials. Therefore, the total number of tracings (n) analyzed at each potential varied. Statistical analysis was performed on at least n = 6. Differences were considered statistically significant at values with P < 0.05. Comparisons plotted on vertical bar graphs represent means ± SE (SigmaPlot 2001).

RESULTS

Effects of insulin by single channel analysis. Figure 1 shows the results of insulin application on BK in cell-attached patches of cultured human MC. BK were identified by the magnitude of the currents and the increased N_po on depolarizing holding potentials. The P_o could be determined by counting the current levels in a patch after excision in 1 mM Ca^{2+} solution and varying the holding potential. As shown by the representative tracings (Fig. 1A), the application of insulin increased the BK

A Total Cellular

B Membrane

Fig. 3. Representative experiments with summary bar plots of immunoblot determinations of effects of insulin on BK-α protein in total cellular (A; n = 3) and membrane (B; n = 3) fractions. *Significance at P < 0.05 using t-test for unpaired data.
activity at a holding potential of −40 mV. Although this same relative increase in $P_o$ was observed at all holding potentials, most data for comparison were accumulated at $V_p = −40$ mV, $V_p$ is pipette potential. The bar plots of Fig. 1, B and C, illustrate that insulin treatment increased the $P_o$ and the overall percentage of seals (≥5 GΩ) that contained BK, respectively. As shown by the $I$-$V$ relations for BK in Fig. 1D, the extrapolated reversal potential for BK shifted to the right in the insulin-treated cells, indicating that membrane potential hyperpolarized with insulin treatment. A hyperpolarizing membrane potential is consistent with a global increase in BK activity in the plasma membrane. The slopes of the $I$-$V$ relations for BK in insulin-treated and control cells were 140 and 159 pS, respectively.

**Effects of insulin on BK RNA and protein expression.** Real-time PCR was used to determine whether BK-α transcript was increased with insulin treatment. As shown in Fig. 2, the mRNA for BK-α increased by more than fivefold in the cells treated with insulin.

As shown in Fig. 3A with representative immunoblots and the summary bar plots summarizing the densitometry of the protein, the relative expression of total cellular BK-α increased substantially in the insulin-treated cells. The membrane fraction (Fig. 3B) exhibited an insulin-evoked increase of ∼35% in BK-α expression. The ratio of the membrane to cytosolic fraction with insulin treatment was 0.26 ± 0.02, a value that was not significantly different from control, which was 0.23 ± 0.02.

It was demonstrated previously by RT-PCR and Western blot in this laboratory that human MC in culture express BK-β1-subunits (24). As shown by the immunoblots and the summary bar plot of Fig. 4, the relative expression of BK-β1 increased in the insulin-treated cells. Thus the relative expression of both BK-α and BK-β1 was increased by approximately fourfold.

**Role of MAPK signaling.** We used the MAPK inhibitors PD-098059 and U-0126 to determine the effects of MAPK signaling on the insulin-stimulated increase in BK-α. As shown in Fig. 5A, neither PD-098059 nor U-0126 affected the basal levels of BK-α expressed in total cellular protein. However, when cells were insulin treated, the insulin-induced increase in BK-α expression was significantly attenuated by both PD-098059 and U-0126 (Fig. 5B). Figure 6 compares the basal and insulin-stimulated effects of MAPK inhibitors on BK-α expression in the membrane fraction. Neither PD-098059 nor U-0126 affected the basal levels of BK-α expression in the plasma membrane.
membrane fraction (Fig. 6A). However, both MAPK-inhibiting agents attenuated the insulin-induced increase in BK-α expression in the membrane fraction (Fig. 6B).

The Western blot of Fig. 7 shows the effects of insulin and insulin with PD-098059 on the phosphorylation of ERK1/2 in human MC. As shown by the summary bar plots of the relative densitometry values, insulin evoked an 82% increase in ERK1/2 phosphorylation. PD-098059 significantly reduced phosphorylated (p)-ERK1/2 to a relative value below control and insulin-treated levels. The quantity of p-ERK1/2 expression was normalized to unphosphorylated ERK1/2, which did not change with insulin treatment. It is concluded that the insulin-induced increase in BK-α expression is at least partially due to activating the MAPK pathway.

**Effects of IGF-I.** We determined whether IGF-I also increased BK-α mRNA and protein expression. The dissociation constant for IGF-I binding to IGF-IR on MC is between 2 and 3.2 nM (2). Figure 8 shows that 5 nM of IGF-I analog increased significantly both BK-α mRNA (Fig. 8A) and protein (Fig. 8B) expression in human MC.

**Expression of BK-α in an in vivo hyperinsulinemic model.** Previously, we showed that C57Bl/6 mice fed a high-fat diet exhibited profound obesity and hyperinsulinemia (46). We used this model to determine whether glomeruli from the hyperinsulinemic mice exhibit increased BK-α expression. After providing mice a high-fat diet for 13 wk, the plasma insulin concentration increased significantly ($P < 0.05$) from $0.53 \pm 0.26$ (n = 4) to $4.4 \pm 2.1$ (n = 5) ng/100 ml. As shown by the immunohistochemical stainings of Fig. 9A, BK-α was expressed with greater intensity in glomerular cells of kidneys harvested from the obese mice. Although positive identification of the glomerular cell types was not made, it appeared as though both podocytes and MC exhibited increased staining of BK-α. Figure 9B, a bar plot summary of real-time PCR experiments, reveals a significant increase in BK-α mRNA in the glomeruli from mice on a high-fat diet. As shown by the representative Western blot and summary of the densitometry measurements (Fig. 9C), BK-α was expressed in a significantly greater quantity in the glomeruli from the mice fed the high-fat diet.

**DISCUSSION**

The results of this study showed that insulin, via stimulation of the MAPK pathway, increased the activity of BK in the plasma membrane of human MC. The abundance of BK in the plasma membrane was elevated due to increased protein synthesis of BK-α and BK-β1. Stimulation of BK by insulin hyperpolarizes the plasma membrane and could partially explain the hyperfiltration and mesangial expansion in the early stages of type 2 DM (6, 8, 10, 45).

Consequences of an insulin-evoked increase in mesangial BK. An insulin-evoked increase in BK activity agrees with a previous study that demonstrated an attenuation by insulin of
ANG II- and endothelin-1-evoked mesangial contraction with Ca\(^{2+}\) transients (20). In that study, the reduction of the Ca\(^{2+}\) transients by insulin was eliminated on removal of extracellular Ca\(^{2+}\), suggesting that the Ca\(^{2+}\) increase was the result of Ca\(^{2+}\) entering the cell via plasmalemmal Ca\(^{2+}\) channels. MC contain at least two types of plasmalemmal Ca\(^{2+}\) channels, store-operated Ca\(^{2+}\) channels comprised of canonical transient receptor potential ion channel, subtype 4 (TRPC4) (43, 44) and TRPC1 (35) and Ca\(^{2+}\) channels that are dihydropyridine and voltage sensitive (16, 17). The inhibition of mesangial contraction and Ca\(^{2+}\) transients can be explained by insulin activating BK, which would hyperpolarize the plasma membrane potential and reduce Ca\(^{2+}\) influx via voltage-gated Ca\(^{2+}\) channels.

We did not investigate the role of the BK-\(\beta_1\)-subunit in the insulin-evoked activation of BK. It is known that BK-\(\beta_1\), which was upregulated by insulin in this study, enhances the Ca\(^{2+}\)/voltage sensitivity of BK-\(\alpha\) (26, 29). It is possible that the increased \(P_o\) of BK in insulin-treated cells could be explained by the notion that, under control conditions, human MC express BK-\(\alpha\) without the BK-\(\beta_1\)-subunit, and insulin induces the association of BK-\(\beta_1\) with the BK-\(\alpha\) in the plasma membrane. However, Toro et al. (41) observed increased endocytic retrieval of BK-\(\alpha\) from the plasma membrane of HEK 293 cells when coexpressed with the BK-\(\beta_1\)-subunit.

Activation of BK by insulin resulted in a shift in reversal potential in the \(I-V\) relation from 32 to 47 mV, demonstrating that activation of BK hyperpolarized the membrane potential by 15 mV. A hyperpolarizing membrane potential would increase a driving force for Ca\(^{2+}\) entry via TRPC4/TRPC1 channels. Cell Ca\(^{2+}\) entry could further activate BK, complet-

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**Fig. 8.** A: effects of insulin-like growth factor I (IGF-I) analog on mesangial BK-\(\alpha\) mRNA expression (\(n = 3\)). B: immunobots with summary bar plots illustrating the effects of IGF-I analog on BK-\(\alpha\) protein expression (\(n = 4\)).

**Fig. 9.** A: immunohistochemical staining revealed increased BK-\(\alpha\) expression in the glomeruli of mice fed a high-fat diet (right) compared with control animals (left). B: summary bar plots reveal an increase in BK-\(\alpha\) mRNA. C: representative Western blot with summary bar plots show an increase in BK-\(\alpha\) protein in the mice fed a high-fat diet.

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Stimulation of BK via IGF-IR and MAPK. The principal role of insulin is to control intracellular metabolism, whereas IGF-I mediates growth and differentiation. However, insulin stimulation of IGF-IR causes mitogenesis of VSMC (4), and both insulin and IGF-I stimulate growth of hepatic stellate cells (37). Insulin and IGF-I have similar mitogenic properties because their respective receptors have very similar protein structures. However, MC have a minimal quantity of IR but synthesize IGF-I and have a large quantity of IGF-IR (2, 3). Therefore, glucose metabolism in MC is normally independent of basal or even postfeeding levels of insulin. In type 2 DM, plasma insulin is often elevated to levels that are 20-fold more than the normal range (38). Thus 100 nM is not outside the realm of pathophysiologically concentrations of insulin, particularly because the exposure to insulin in type 2 DM is for a much longer duration than in the cultured cell model system. It should be mentioned that Abrass et al. (2) found that a much higher concentration of insulin (1 μM) was required to achieve the same effect as 1 nM IGF-I to stimulate proliferation of rat MC. However, human and rat MC may be different with respect to insulin sensitivity.

Although several pathways downstream of IGF-IR are potentially activated by insulin, we focused on the MAPK pathway. In neuronal hippocampal cells, insulin stimulated BK via MAPK signaling, which enhances the Ca2+ sensitivity of BK but does not increase the basal intracellular Ca2+ concentration (32, 46). Schrader et al. (34) demonstrated that ERK/MAPK directly activated Kv4.2 by phosphorylating T607. Interestingly, phosphorylation of Kv4.2 by MAPK required the presence of the associated accessory subunit, KChIP (34).

Although MAPK stimulation did not increase the basal intracellular Ca2+ concentration in hippocampal cells (32), there may be an insulin-induced increase in MAPK that results in a subplasmalemmal Ca2+ concentration that is high enough to activate BK. In support of this notion, IGF-I stimulated the translocation of an expressed TRP cation channel to the plasma membrane of Chinese hamster ovary cells (21). Moreover, both insulin and IGF-I upregulated stably expressed vanilloid transient receptor potential ion channel, subtype 1 in neuroblastoma cells by mechanisms involving both phosphatidylinositol 3-kinase and MAPK (25). Therefore, it is possible that activation of BK by insulin is the indirect result of increasing expression of plasmalemmal TRP channels, which deliver undetected quantities of subplasmalemmal Ca2+ to BK.

In vivo evidence for insulin-stimulated BK. Plasma insulin is one of several molecules that are elevated in association with metabolic syndrome and early stage type 2 DM. We have shown with a type 2 DM mouse model that elevated insulin is associated with an increase in glomerular BK-α. However, this increase could be the result of other compounds, like aldosterone, which is elevated in type 2 DM mice (27). Indeed, a previous study has shown that renal BK-α mRNA (28) and BK-mediated K+ secretion in the distal nephron (5) is upregulated by a high-K+ diet, which stimulates aldosterone in mammals. Therefore, aldosterone may independently increase BK activity in MC in vivo.

In summary, we have found that high plasma levels of insulin increase BK activity in human MC in vitro, in part, by stimulating the MAPK pathway. The effects of insulin may explain the increased BK-α observed in glomeruli from type 2 DM mice and may partly explain the hyperfiltration observed in the early stages of type 2 DM.

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