Phosphatidylinositol 3'-Kinase-dependent Activation of Renal Mesangial Cell Ki-Ras and ERK by Advanced Glycation End Products*

Advanced glycation end products (AGEs) are produced by the non-enzymatic glycation of proteins and lipids. AGE levels are pathologically elevated in a number of inflammatory diseases and in diabetes mellitus. There is evidence that AGEs, acting through the receptor for AGEs, contribute to diabetic complications. Nephropathy is a major complication of diabetes mellitus. However, the initiating molecular events that trigger diabetic renal disease are unknown. Renal mesangial cells produce excess extracellular matrix in response to treatment with transforming growth factor-β, and excess mesangial cell matrix production, by impairing glomerular filtration, contributes to diabetic nephropathy. AGEs are known to trigger the autocrine production and release of transforming growth factor-β. However, it is unclear how AGEs signal in mesangial cells. Here we show that treatment of mesangial cells with AGEs and with the receptor for AGEs agonist S100 triggers activation of the extracellular signal-regulated kinase (ERK) and phosphatidylinositol 3'-kinase (PI3K) pathways. AGEs trigger the GTP loading of mesangial cell Ras, and AGE activation of ERK requires Ras. We observe that Ki-Ras, but not Ha-Ras, is the target of AGE action. Surprisingly, inhibition of PI3K blocks both ERK and Ki-Ras activation. We also observe that activation of ERK and the PI3K target kinase protein kinase-B is blocked with free radical scavengers, indicating a role for reactive oxygen species in AGE recruitment of PI3K. Thus, AGEs signal to Ki-Ras and ERK through reactive oxygen species-dependent activation of PI3K.

The receptor for advanced glycation end products (RAGE) is a type-1 transmembrane protein of the immunoglobulin superfamily. RAGE interacts with a surprisingly diverse variety of extracellular ligands. Chief among these are advanced glycation end products (AGEs) (1).

AGEs are produced by a non-enzymatic, Maillard reaction between reducing sugars and either proteins or lipids. AGEs accumulate slowly with normal aging and at a much accelerated rate in a variety of inflammatory conditions and diabetes mellitus. The elevated accumulation of AGEs has been linked to the pathophysiology of diabetic peripheral vascular disease, and the AGE-RAGE interaction has been shown to play a central role in the development of diabetic vasculopathy (2–5).

Diabetes is the leading cause of end-stage renal failure, and much of the morbidity and mortality of diabetes can be attributed to nephropathy. Diabetic nephropathy is associated with the pathophysiologic deposition of extracellular matrix proteins into the glomerular basement membrane. This matrix deposition is carried out largely by renal mesangial cells. A significant consequence of excess matrix deposition is increased glomerular filtration pressure. Renal mesangial cell hypertrophy and glomerulosclerosis ensue, resulting ultimately in a loss of renal filtration function (6). There is good evidence that AGEs are important to the early events in diabetic nephropathy. The dysfunctional production of matrix proteins by mesangial cells is triggered largely by transforming growth factor-β (TGF-β). Administration of AGEs to cultured glomerular endothelial cells leads to the elevated autocrine expression of mesangial cell TGF-β that, in turn, could stimulate matrix protein production by neighboring mesangial cells in the glomerulus (7). Moreover, transgenic overexpression of RAGE in a mouse line prone to diabetes leads to the rapid development of renal disease by a process that can be reversed with AGE inhibitors (8).

Still, little is known of how AGEs, through RAGE, signal in mesangial cells, an important consideration if the early events of diabetic nephropathy are to be understood. The intracellular extension of RAGE is small and has no apparent catalytic function (1). However, mutant RAGE proteins lacking the cytoplasmic domain are inactive and can function as dominant inhibitors of AGE signaling (9). Thus, either RAGE signals through its intracellular extension, or this extension is required for RAGE to recruit a coreceptor with signaling properties. Activation by AGEs of the Janus kinases, nuclear factor κB (NFκB) and mitogen-activated protein kinases (MAPKs), including the extracellular signal-regulated kinases (ERKs) and c-Jun NH2-terminal kinases/stress-activated protein kinases (JNKs/SAPKs), has been reported, as has activation of Ras (9–12). However, it is not known how RAGE couples to these pathways.

Here we show that the mesangial cell ERK and phosphatidylinositol 3'-kinase (PI3K) pathways are activated by AGEs.
PI3K-dependent Ras/ERK Activation by AGEs

JNK/SAPKs and p38 MAPKs are not activated in these cells. We also show that the GTP loading of mesangial cell Ras is stimulated by AGEs and that ERK activation is Ras-dependent. We find that mesangial cell Ki-Ras, but not Ha-Ras, is recruited by AGEs. PI3K is a Ras effector in most, but not all, signaling pathways (13), with MAPK and PI3K effectors functioning largely in parallel to execute cellular signaling programs. Surprisingly, however, we find that activation of both mesangial cell Ras (Ki-Ras) and ERK requires PI3-kinase. We also find that ERK and PI3K activation by AGEs is dependent upon the generation of reactive oxygen species (ROS) and on non-receptor Tyr kinases (NRTKs) of the Src family. Our results suggest that AGEs exploit an unusual PI3K-dependent mechanism for signaling to monomeric GTPases and MAPKs.

EXPERIMENTAL PROCEDURES

Cells, Treatments, and Reagents—Renal mesangial cells were isolated from the glomeruli of male Sprague-Dawley rats (125–175 g) as described (14). Cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 5 mM-lglutamine, and penicillin streptomycin solution (100 units/ml and 100 μg/ml, respectively). Integrity of the mesangial cell phenotype was confirmed regularly by immunoblotting with anti-smooth muscle actin and Thy-1 antibodies. For AGE and other treatments, cells were serum-starved for 48 h and treated with AGE (100 μg/ml), sorbitol (300 μM), S100 (5 μg/ml), EGF (100 ng/ml), endothelin-1 (ET-1, 100 nM), or H2O2 (0.1 mM) for either 15 min or for the 0.5% Triton X-100, 10% glycerol, 0.5 mM phenylmethylsulfonyl fluoride, 100 units/ml and 100 μg/ml), sorbitol (300 μM), S100 (5 μg/ml), EGF (100 ng/ml), endothelin-1 (ET-1, 100 nM), or H2O2 (0.1 mM) for either 15 min or for the indicated times as shown in the figures. As indicated, cells were pretreated with 100 nM U0126, 1 μM LY294002, 10 μM PDTC, 50 μM ammonium pyrrolidinecarbodithioate (PDTC), 5 μM diphenyleneiodonium chloride (DPI), 50 μM 4,4′-isopropyldenedi-thio-bis(2,6-butyl)-phenol (protocub), or 20 μM 4-amino-5-(4-chloro-phenyl)-7-(t-butyl)-pyrazolo[3,4-d]-pyrimidine (PP2) for 30 min in some experiments. PDTC and DPI were from Sigma, EGF, ET-1, S100, U0126, LY29402, wortmannin, probucol, and PP2 were from Calbiochem. AGE was prepared from crystallized, lyophilized bovine serum albumin and glucose as described (7). A recombinant adenovirus encoding M2-FLAG-N17-Ras was prepared as described (15). Mesangial cells were infected at a multiplicity of infection of 200, with virus 12 h before treatment with S100.

Kinase Assays—ERK and JNK/SAPK immune complex kinase assays were performed as described (16). Myelin basic protein or glutathione S-transferase (GST)-c-Jun (1–135), respectively, were used as substrates. In addition, in vivo phosphorylation of the activating regulatory phosphoacceptor sites on ERK-1 and -2, p38 MAPK, and protein kinase B (PKB) was assayed with the relevant phospho-specific antibodies. Thus, cells were lysed in 500 μl of SDS-PAGE loading buffer. Equal amounts of total protein were subjected to SDS-PAGE and immunoblotting as described (16, 17). We used antibodies against specific phosphoacceptor residues for ERK2 (Thr-202/Tyr-204), (Thr-180/Tyr-182), and PKB (Thr-473), all from Cell Signaling Technology. In parallel, we employed rabbit anti-total ERK (Santa Cruz Biotechnology), p38 (Santa Cruz Biotechnology), and PKB (Cell Signaling Technology).

Ras GTP Loading Assays—The activation of Ras was detected as the acquisition of the ability to bind the immobilized Ras binding domain of Ras effector Raf-1 (amino acids 51–131), expressed as a GST fusion protein and bound to glutathione-agarose (18). Thus, cells were treated with the agonists indicated in the figures, and lysates were prepared in a buffer designed to maintain the guanine nucleotide binding status of Ras. An aliquot of extract was treated with high glucose (30 mM) for 24 h. Cells were then treated with S100 for the indicated times. 100 μg of extract were subjected to SDS-PAGE and immunoblotting with anti-P-PKB or anti-total PKB as indicated. A, rat renal mesangial cells were treated for 15 min with ET-1, S100, or AGE as indicated, at the concentrations described under “Experimental Procedures.” Cell lysates were prepared and normalized to equal protein concentration. 100 μg of extract were subjected to SDS-PAGE and immunoblotting with anti-P-PKB or anti-total PKB as indicated. B, mesangial cells were treated with S100 for the indicated times. 100 μg of extract were subjected to SDS-PAGE and immunoblotting with anti-P-PKB or anti-total PKB as indicated.

RESULTS AND DISCUSSION

Activation of ERK and PI3K by AGE and the RAGE Agonist S100— RAGE interacts with a diverse collection of extracellular stimuli, including AGEs, amphoterin, and the calgrulin S100 (1). We were interested in identifying signaling pathways activated by more than one RAGE agonist. PI3K and its effectors are essential to the control of cell survival, cell size, proliferation, and metabolism. Of note, PI3K effectors have been implicated in cardiomyocyte and mesangial cell hypertrophy (17, 19, 20). The products of PI3K activity are absolutely required for phosphorylation at Ser-308 and -473 and consequent recruitment of a functional kinase that targets PKB Ser-473 (21–23).

The phosphorylation of PKB is a facile readout for activation of the PI3K pathway. AGE and S100 treatment leads to rapid phosphorylation and activation of rat renal mesangial cell PKB as detected on immunoblots probed with antibodies directed toward PKB phosphorylated at Ser-473 (Fig. 1A). Glycogen synthase kinase-3 (GSK3) is phosphorylated and inhibited by PKB, an event that is associated with the recruitment of many transcriptional programs themselves inhibited by GSK3. We also observe that S100 triggers the phosphorylation of GSK3β (at Ser-9), indicating recruitment of a functional PI3K→PKB→GSK3 pathway (Fig. 1A). Consistent with a role for PI3K, PKB activation by either AGE or S100 is blocked with wortmannin, a highly specific inhibitor of PI3K (13) (Fig. 1A).

Hyperglycemia has been implicated as a trigger for renal mesangial cell pathology in diabetes. Treatment of mesangial cells with elevated glucose has been shown to activate immediate early gene expression and to potentiate the activation of signaling pathways by other stimuli (24). Consistent with this, we find that culture of mesangial cells for 24 h in 30 mM glucose significantly activates the PI3K pathway. S100 further enhances glucose activation of PKB. Glucose has no effect on PKB levels (Fig. 1B).

AGE and S100 treatment of mesangial cells also elicits substantial activation of the ERK subgroup of MAPKs (Fig. 2A). Activation is comparable with that incurred by ET-1. By contrast, neither AGE nor S100 activates the mesangial cell JNK/SAPK or the p38 MAPKs under conditions wherein, respectively, ET-1 and sorbitol produce robust activation of these.
MAPKs (Fig. 2, B and C). As with PI3K signaling, basal and S100-stimulated ERK phosphorylation and activation are modestly elevated by increasing the glucose concentration in the media from 10 to 17 mM for 24 h; this elevation is catalytic inasmuch as ERK polypeptide levels are not affected by higher glucose (Fig. 3, A and B).

Thus, both AGE and S100 activate identical signaling pathways in mesangial cells, and S100 is a reasonable RAGE agonist in these cells. In further support of this, activation of ERK and PKB by both AGE and S100 can be completely inhibited with the PI3K inhibitor wortmannin (Figs. 1A, 2A, and 3; discussed below).

**RAGE-mediated ERK Activation Is PI3K- and Ras-dependent**—Canonical signaling pathways emanating from receptor tyrosine kinases and trimeric G protein-coupled receptors typically require the monomeric G protein Ras (25). GTP loading and consequent activation of Ras leads to recruitment of effectors that include PI3K and the Rafs, MAPK-kinase-kinases upstream of ERK. Raf/ERK and PI3K are then thought to execute parallel, largely discrete, signaling pathways that evoke the relevant cellular responses (13, 25). There are, however, exceptions to this paradigm. For example, constitutively active PI3K can trigger Ras GTP loading in *Xenopus* oocytes (26), and signaling to Ras and ERK from the B cell receptor can be completely blocked with PI3K inhibitors (27).

Interestingly, we find that mesangial cell ERK phosphorylation and activation by AGE are substantially inhibited by either wortmannin (Fig. 3, B and C and Fig. 6A) or LY294002, a second highly specific PI3K inhibitor (13) (Fig. 6C). These results suggest that AGEs trigger PI3K-dependent ERK activation. ET-1 and EGF activation of ERK are unaffected by wortmannin (Fig. 3C), and EGF activation of ERK is unaffected by LY294002 (Fig. 6C). Thus, in mesangial cells, PI3K-dependent ERK activation is a phenomenon relatively specific to AGE signaling and is not a feature of receptor tyrosine kinase (EGF receptor) or trimeric G protein-coupled receptor (ET-1 receptor) signaling in mesangial cells.

PI3K is both an effector for Ras and, as noted above, is in some systems a contributor to Ras activation (13, 26, 27). To explore the role of Ras in AGE activation of mesangial cell ERK, we used a recombinant adenovirus that expresses dominant inhibitory (N17)-Ras. From Fig. 4 it is clear that in mesangial cells infected with this virus, S100 no longer activates ERK. ERK activation is unaffected by infection with a control (β-galactosidase) virus. Thus, RAGE signals to ERK through a Ras and PI3K-dependent mechanism.

**RAGE Activation of Ras Is PI3K-dependent; RAGE Selectively Recruits Ki-Ras**—Monomeric G proteins of the Ras superfamily are inactive in the GDP-bound state and active in the GTP-bound state. Ras proteins have a slow intrinsic GTPase activity that is accelerated by GTPase activating proteins. Activation of Ras proteins is mediated by guanyl nucleotide exchange factors that accelerate GDP release. Inasmuch as GTP is in excess in the cell, GDP release is followed swiftly by GTP binding (25). Active Ras proteins acquire the ability to bind effector proteins (25); accordingly, detection of this binding can serve as a convenient method for monitoring Ras protein activation. The MAP3K Raf-1 is an established effector for Ras. Binding of Raf-1 to Ras results in Raf-1 membrane translocation and is a key rate-limiting step in Raf-1 activation (25).
FLAG-N17-Ras were detected in parallel immunoblots with the cognate immunoblotting with anti-phospho-ERK antibody. Total ERK and NAc is without effect on ET-1 activation of PKB (Fig. 5). ERK likely requires the production of ROS. Interestingly, the ERK activation by AGE is completely inhibited by NAc, indicating that this signaling mechanism too involves the production of ROS.

Amino acids 51–131 of the Raf-1 polypeptide contain the domain involved in GTP-dependent Ras binding (26). A GST-Raf-1 (51–131) fusion protein, immobilized on glutathione-agarose, can selectively trap GTP-Ras produced in cells consequent to extracellular signals and can be used to follow the stimulus-dependent accumulation of activated Ras (18).

We treated mesangial cells with S100, EGF, or ET-1. Cell lysates were prepared and incubated with immobilized GST-Raf-1 (51–131). From Fig. 5A, it is evident that EGF triggers a strong activation of Ras detectable as Ras immunoreactivity recovered on the Raf-1 (51–131) beads. S100 and ET-1 also stimulate substantial Ras activation. Ras activation by S100 is comparatively transient, reaching a maximum at 2 min (Fig. 5B). We also observe that Ras activation by S100 is completely reversed with wortmannin, suggesting AGE recruits Ras through a PI3K-dependent mechanism (Fig. 5B).

Of the three Ras isoforms present in mammalian cells (Ha-Ras, Ki-Ras, and N-Ras), Ki-Ras has the most profound effect on cell proliferation and overall physiology. Thus, disruption of ki-ras is embryonic lethal, whereas disruption of ha-ras produces no obvious phenotype (28). Similarly, most human cancers that involve a ras mutation have mutations in ki-ras (29).

We asked whether AGE selectively activated Ki- or Ha-Ras (N-Ras expression is not detectable in renal mesangial cells; data not shown). Using a similar assay to that described above for total Ras (this time substituting specific Ki- and Ha-Ras antibodies for the Pan-Ras antibody used in Fig. 5, A and B), we find that GTP loading of Ki-, but not Ha-Ras, is stimulated by S100, AGE, and EGF in renal mesangial cells (Fig. 6A). As with ERK activation (Fig. 3), S100 and AGE activation of Ki-Ras is reversed with wortmannin (Fig. 6A) and LY294002 (Fig. 6C). By contrast, EGF and ET-1 activation of Ki-Ras is not prevented by wortmannin, and EGF activation of Ki-Ras is not inhibited by LY294002 under conditions in which activation of PKB is completely inhibited (Fig. 6, B and C).

RAGE, but Not ET-1, Signaling to ERK and PI3K/PKB Requires the Generation of ROS—It has been reported that engagement of RAGE triggers ROS production (1, 29). ROS have been implicated in signaling (30, 31). Thus, proinflammatory cytokines and mitogens can, in some cells, trigger a burst of ROS production that is necessary for subsequent MAPK activation (30). NAc is a potent free radical scavenger that can dampen ROS-dependent signaling. We find that NAc (20 mM) can efficiently inhibit S100 activation of both PKB and ERK; ET-1 activation of ERK is at best modestly inhibited by NAc, whereas NAc is without effect on ET-1 activation of PKB (Fig. 7, A and B). Thus, AGE signaling to mesangial cell PI3K and ERK likely requires the production of ROS. Interestingly, the activation of ERK and PI3K incurred by high glucose (20 mM) is also blocked by NAc, indicating that this signaling mechanism too involves the production of ROS.

To confirm ROS-dependent AGE activation of ERK, we next employed, at lower concentrations than that used for NAc (see “Experimental Procedures”), three additional ROS scavengers: PDTC, a second general antioxidant, DPI, an inhibitor of the NADPH oxidase family, and probucol, which scavenges lipid peroxides. Under conditions in which 20 mM NAc affected near complete inhibition of S100 activation of ERK, PDTC and DPI, employed at 50 and 5 mM, respectively, also reduced S100 activation of ERK. By contrast, probucol was without effect (Fig. 7C). We conclude from the data in Fig. 7A–C that S100 activation of ERK is ROS-dependent and that the source of ROS is not likely to be lipid peroxides. AGE-stimulated ROS may instead arise from oxidases related to the NADPH oxidase. This finding is consistent with results reported elsewhere indicating a role for NADPH oxidases in AGE signaling (29).

Exogenous hydrogen peroxide is frequently employed as a model for the endogenous ROS produced in response to engagement of different cellular receptors. Indeed, we find that peroxide added exogenously activates mesangial cell PKB and ERK. However, in contrast to AGE signaling, ERK activation by peroxide is not reversed with LY294002 under conditions wherein PKB activation is (Fig. 7D). Thus, although AGEs trigger ROS production (1, 29) and AGE activation of ERK is clearly ROS-dependent, exogenous peroxide does not simulate the mechanism by which the endogenous ROS is produced in response to AGE signal to ERK.

There is evidence that the activation of PKB by certain exogenous sources of ROS requires NRTKs of the Src family (32). Accordingly, we wished to evaluate the potential role for Src family NRTKs in AGE activation of ERKs. PP2 is a potent and specific inhibitor of NRTKs of the Src family (33). We find that PP2 completely inhibits S100 activation of ERK, indicating that AGEs recruit Src family NRTKs to signal to ERK (Fig. 7C).

Our results indicate an unusual mechanism of S100-dependent mesangial cell ERK activation. We find that S100 triggers the activation of Ki-Ras and PI3K and that activation of Ki-Ras and its effector ERK requires prior activation of PI3K. Both PI3K and ERK activation, in turn, require the production of ROS, and S100 activation of ERK requires a NRTK. Inasmuch as Src has been shown to couple ROS to PI3K/PKB (32) and we find that Ras activation by AGE requires prior activation of PI3K, we propose that AGEs trigger ROS-dependent activation of NRTK(s) which,
in turn, recruit PI3K. PI3K then activates Ki-Ras (Fig. 8). This contrasts with more canonical signaling pathways wherein PI3K is a Ras effector and subsequent signaling from PI3K is largely discrete from MAPK signaling (13, 25).

Mesangial cell AGE signaling differs significantly from that reported elsewhere. We find no activation of JNK/SAPK or p38 by AGE or S100, contrary to results from other cell types (12) nor do we observe activation of mesangial cell NF\textsubscript{κ}B by AGE.\textsuperscript{2} The PC12 cell NF\textsubscript{κ}B is activated by AGE (9). Thus, AGE signaling in mesangial cells is more restricted than that elicited by ET-1 (which recruits JNK/SAPK and p38 MAPK) (17). In this regard, it is noteworthy that we find that AGE does not stimulate mesangial cell hypertrophy, as occurs in diabetes (6).

\textsuperscript{2} D. Xu and J. M. Kyriakis, unpublished observations.
Our observations indicate that this process requires activation of JNK/SAPK as well as ERK and PI3K (17).

How PI3K might recruit Ras is unclear. Many Ras guanine nucleotide exchange factors contain pleckstrin homology (PH) domains (31). A subset of PH domains can bind the products of PI3K activity (13), and it is conceivable that one or more such Ras exchange factor(s) is a target for RAGE-activated PI3K. How RAGE recruits its immediate effectors remains unclear.

Transfection studies using truncated forms of RAGE indicate that RAGE signaling to NF-kB in neuroblastoma cells requires the RAGE intracellular extension, and expression of truncated RAGE missing the intracellular extension can block signaling through intact, endogenous RAGE (9). The RAGE intracellular extension has no obvious molecular features, other than an acid-rich stretch of amino acids reminiscent of proline-glutamic acid/aspartic acid-serine-threonine-rich (PEST) motifs (1). PEST domains have been implicated in protein turnover by the ubiquitin proteasome pathway (34). As yet there is no evidence for RAGE ubiquitination and proteasomal degradation. It is possible that RAGE behaves like the receptors for proinflammatory cytokines. Receptors such as that for tumor necrosis factor (TNF) possess no intrinsic enzymatic activity and instead exist as preformed oligomers, the conformation of which changes with ligand binding (35). A consequence of this change is the recruitment of intracellular adapter proteins such as the TNF receptor-associated factors (TRAFs) that mediate signaling (30, 35). By the same token, AGEs may induce RAGE as preformed oligomers, the conformation of which pre-existing RAGE oligomers. This, in turn, would prompt the binding and activation of intracellular effector molecules similar to the TRAFs. These adapters may include polypeptides that trigger ROS production and consequent NRTK and PI3K activation. It is equally possible that RAGE engagement recruits a co-receptor that mediates signaling, a mechanism similar to signaling by TGF-β and bacterial lipopolysaccharide (LPS) receptor toll-like receptor-4 (TLR4). Thus, the type-2 TGF-β receptor binds agonist, an event that stimulates the type-2 receptor’s association with, phosphorylation, and activation of the type-1 TGF-β receptor. The type-1 receptor then transduces the TGF-β signal (36). Similarly, although TLR4 is the signaling receptor for LPS, LPS must first bind CD14, a cell surface coreceptor, in order to engage TLR4 (37).

Early molecular events in the pathophysiology of diabetic kidney are poorly understood. Elevations in blood glucose alone are thought to trigger signaling events that initiate diabetic nephropathy. Thus, it has been shown that high glucose activates the activator protein-1 transcription factor. In addition, the release of TGF-β, a critical early step in diabetic nephropathy linked to the production of excess extracellular matrix proteins, can also be stimulated in mesangial cells by elevated glucose in the culture media (6, 7, 24). We also see activation of both the ERK and PI3K pathways by high glucose, but it is important to note that at glucose concentrations consistent with those observed in diabetes (no greater than 20 mM), the degree of activation of both pathways (ERK especially) is relatively modest in comparison with that incurred by other stimuli, including ST100. It is conceivable that, over an extended time and in conjunction with other chronic stress stimuli, this weak activation could contribute significantly to nephropathy. It is also conceivable that a substantial portion of the effect of glucose in vivo could be mediated by AGEs. Thus, RAGE over-expression in diabetic mice provokes nephropathy (8), and treatment of mesangial cells with AGEs triggers TGF-β expression (7). Moreover, serotonin induction of TGF-β in mesangial cells requires ERK (38), and it is possible that ERK activation by AGE may contribute to TGF-β expression.

The importance of PI3K and MAPKs to the pathology of chronic disease is becoming apparent. Thus, the PI3K, ERK, and JNK/SAPK pathways are necessary for both ET-1-induced cardiomyocyte and renal mesangial cell hypertrophy (15, 17, 19, 20). Our results indicate a role for PI3K and ERK in AGE signaling. Given the importance of AGE to matrix deposition in diabetes (1–5, 7, 8), it will be important to determine whether ERK and PI3K are important to the AGE induction of matrix protein deposition and to other features of diabetic nephropathy.

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