Role of inhibitory κB kinase and c-Jun NH$_2$-terminal kinase in the development of hepatic insulin resistance in critical illness diabetes

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Jiang S, Messina JL. Role of inhibitory κB kinase and c-Jun NH$_2$-terminal kinase in the development of hepatic insulin resistance in critical illness diabetes. Am J Physiol Gastrointest Liver Physiol 301: G454–G463, 2011. First published June 16, 2011; doi:10.1152/ajpgi.00148.2011.—Hyperglycemia and insulin resistance induced by acute injuries or critical illnesses are associated with increased mortality and morbidity, as well as later development of type 2 diabetes. The molecular mechanisms underlying the acute onset of insulin resistance following critical illness remain poorly understood. In the present studies, the roles of serine kinases, inhibitory κB kinase (IKK) and c-Jun NH$_2$-terminal kinase (JNK), in the acute development of hepatic insulin resistance were investigated. In our animal model of critical illness diabetes, activation of hepatic IKK and JNK was observed as early as 15 min, concomitant with the rapid impairment of hepatic insulin signaling and increased serine phosphorylation of insulin receptor substrate 1. Inhibition of IKK$\alpha$ or IKK$\beta$, or both, by adenovirus vector-mediated expression of dominant-negative IKK$\alpha$ or IKK$\beta$ in liver partially restored insulin signaling. Similarly, inhibition of JNK1 kinase by expression of dominant-negative JNK1 also resulted in improved hepatic insulin signaling, indicating that IKK and JNK1 kinases contribute to critical illness-induced insulin resistance in liver.

Insulin resistance is involved in the pathogenesis of a number of chronic metabolic disorders, such as type 2 diabetes and obesity, and develops over months, years, or decades. However, insulin resistance can also occur extremely rapidly after injuries, infection, or critical illnesses and is referred to as “diabetes of injury” or, more inclusively, “critical illness diabetes” (3, 8, 29, 44, 46, 47). Critical illness diabetes-induced acute insulin resistance results in elevated blood glucose due to enhanced hepatic glucose production and/or impaired peripheral glucose uptake (29, 44, 46). This acute development of hyperglycemia and insulin resistance predisposes critically ill patients to detrimental outcomes and increased mortality (6, 14, 22, 45, 53). In addition, much like gestational diabetes, recent clinical studies suggest that the acute development of critical illness diabetes after surgery and the concomitant hyperglycemia are strongly correlated with the later development of type 2 diabetes (10). The molecular mechanisms underlying the development of this acute insulin resistance in critical illness diabetes remain poorly understood. Insights into the pathogenesis of acute insulin resistance may provide novel and specific therapeutic strategies for improving recovery and survival of critically ill patients and may suggest interventions for the greater likelihood of later development of type 2 diabetes.

Insulin is a major hormone regulating glucose homeostasis through suppression of glucose production in liver and promotion of glucose disposal in muscle and adipose tissue (38, 41). Insulin binds to the insulin receptor (IR) on the surface of target cells, resulting in IR autophosphorylation (39, 41). Activated IR recruits and phosphorylates IR substrate (IRS) proteins at multiple tyrosine residues, which in turn bind to phosphatidylinositol 3-kinase and activate the kinase Akt and its downstream targets, regulating glucose metabolism (39, 41). Injuries or critical illness is associated with increased intracellular stress and activation of inflammatory pathways (17, 20, 44, 50). Possible contributors to the insulin resistance of critical illness diabetes are serine kinases, such as inhibitory κB kinase (IKK)-β and c-Jun NH$_2$-terminal kinase (JNK), which share the ability to directly induce serine phosphorylation of IRS1, decrease insulin-stimulated tyrosine phosphorylation of IRS1, and inhibit insulin action (13, 16, 55, 58, 59).

The IKK complex is central to the inflammatory NF-κB pathway (15) and consists of two catalytic subunits, IKK$\alpha$ and IKKB, and a regulatory subunit, Nemo. Overexpression of the active form of IKKB in hepatocytes eventually leads to insulin resistance in liver and skeletal muscle (7). Deletion or inhibition of IKKB in liver (5, 7, 13), but not in skeletal muscle (37), attenuates obesity-related chronic insulin resistance. In addition, Nemo is suggested to be necessary in formation of a IKK-IRS1 complex in TNFα-induced insulin resistance (34).

The JNK kinase may also regulate insulin resistance. Total JNK activity is elevated in dietary and genetic (ob/ob) models of obesity, and absence of JNK1 results in improved insulin sensitivity and decreased adiposity in obese mice (16). Suppression of the JNK pathway in liver improves insulin resistance and decreases blood glucose level in genetic models of severely obese (db/db) mice (35). However, nothing is known about the contribution of the IKK and JNK1 kinase pathways to development of the acute insulin resistance that develops in critical illness diabetes. Unlike high-fat diet or genetic models of obesity or type 2 diabetes, which are chronic problems that take many weeks, months, years, or decades to develop, critical illness diabetes develops very rapidly after an injury or infection. There is no a priori reason to believe that critical illness diabetes will have the same or different mechanisms of development. Since it can develop in our animal model of critical illness diabetes within minutes after hemorrhage (23, 24, 27, 28, 43, 52), the mechanisms of development may be very different from those associated with chronic forms of insulin resistance.

In the present studies, we found that hepatic IKK and JNK were activated within minutes after trauma and hemorrhage,
with a time course similar to the rapid impairment of hepatic insulin signaling and increased serine phosphorylation of IRS1. Inhibition of IKK and/or JNK1 kinases in liver improved hepatic insulin signaling and attenuated the hemorrhage-induced increase in IRS1 serine phosphorylation. The present studies suggest that JNK1 and IKK kinases contribute to the acute development of insulin resistance in critical illness dia-

MATERIALS AND METHODS

Animal model of trauma and hemorrhage. All experimental procedures were carried out in accordance with National Institutes of Health guidelines for the care and use of laboratory animals, and the experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. Male Sprague-Dawley rats (200–250 g body wt; Harlan) were housed in animal facilities for ≥1 wk before experiments. Surgical trauma and hemorrhage were performed as described previously (24, 27, 28, 52). Rats were fasted for 18–20 h before the experiment. Rats were anesthetized by inhalation of 1.5% isoflurane, and a 5-cm midline laparotomy was performed. Polyethylene (PE-50) catheters were placed in both femoral arteries and the right femoral vein for bleeding, monitoring of blood pressure, and resuscitation, respectively. Rats were bled rapidly to reach a mean arterial pressure of 35–40 mmHg within 10 min and maintained for 15 min (TH15°), 30 min (TH30°), 60 min (TH60°), or 90 min (TH90°). The trauma-alone (T) groups were subjected to anesthesia and surgical procedures described for the trauma-and-hemorrhage groups but were not hem-

rharaged. In all groups of rats, 5 U of insulin in saline or saline alone was injected into the hepatic portal vein, and tissues were harvested 1 min after injection and quickly frozen in liquid nitrogen. This high dose of insulin ensures the maximal possible induction, even if the liver is insulin-resistant, and ensures sufficient delivery to the liver during hemorrhage.

Recombinant adenoviral vectors. The recombinant adenoviral (Ad) vectors expressing a dominant-negative (DN) mutant (K44M) of IKKα (Ad-DN-HA-IKKα) (9, 56), a DN mutant (K44A) of IKKβ (Ad-DN-HA-IKKβ) (9, 56), and the control Ad vector expressing β-galactosidase (Ad5-nt-LacZ) were purchased from the University of Iowa Vector Core (9). The two IKK mutants were rendered incapable of binding ATP and, thus, lost functional kinase capability. The Ad vector expressing a DN mutant (T183A/Tyr185F) of JNK1 (Ad-DN-JNK1) was purchased from Cell Biolabs (catalog no. ADV-115) and was rendered kinase-dead by these two mutations within the JNK kinase domain. The Ad vectors were amplified in 293A cells and purified twice by cesium chloride gradient ultracentrifugation followed by dialysis. The biological titer [plaque-forming units (pfu)/ml] was determined by the TCID50 (tissue culture infectious dose 50) method (AdenoVactor Vector System) based on the development of a cytopathic effect in 293 cells. Replication-competent Ad contamination was not detected in purified virus stocks as screened by PCR with the primers flanking the E1 region.

Administration of Ad vectors in vivo. Rats were injected with the purified Ad vectors (1010 pfu/rat) via the tail vein. At 4 days postinjection, the animals were subjected to surgical trauma and hemorrhage for 60 min (TH60°), and tissues were harvested for analysis of insulin signaling and kinase activity.

Western blot analysis. Liver tissues from each animal were homog-


ized in lysis buffer as described previously (24, 28). The tissue lysates were centrifuged, and the supernatants were subjected to SDS-PAGE and transferred to nitrocellulose membranes. The mem-

branes were immunoblotted with anti-Tyr972-phosphorylated IR, anti-

Thr183/Tyr185-phosphorylated JNK1/2, and anti-Ser312-phosphory-

lated IRS1. The liver kinase activity and/or IRS1 serine phosphorylation. The present studies (P-IKKα), total IKKα (T-

IKKα), total IKKβ (T-IKKβ), and total ERK (T-ERK) antibodies; the level of total ERK was probed as a loading control. B: representative EMSA. Liver nuclear proteins from several repeated experiments.

Immunohistochemistry. The liver tissues were rinsed in PBS and embedded in HistoPrep frozen tissue embedding medium (Fisher Scientific) and snap-frozen in liquid nitrogen. Frozen liver sections were cryosectioned (10 μm) and subjected to immunohistochemical staining with anti-hemagglutinin (HA) antibody (Cell Signaling, Danvers, MA) and stored at −80°C. Protein concentrations were measured by bicinechonic acid protein assay. Oligonucleotides containing the κb consensus sequence (5′-AGTTGAGGGACTTT-

CCAGGC-3′) were labeled with [32P]CTP using Klenow DNA poly-

merase. The binding reaction was performed as described previously (26). Briefly, nuclear protein (10 μg) was incubated with labeled oligonucleotide probe for 30 min on ice and separated on 4–20% polyacrylamide Tris-glycine-EDTA gels. Excess of a specific unla-

beled competitor sequence was used to out-compete the specific interactions to indicate specific binding.

Gel-shift assay. Nuclear proteins were extracted from frozen liver samples according to the manufacturer’s instruction (Active Motif, Carlsbad, CA) and stored at −80°C. Protein concentrations were measured by bicinechonic acid protein assay. Oligonucleotides containing the κb consensus sequence (5′-AGTTGAGGGACTTT-

CCAGGC-3′) were labeled with [32P]CTP using Klenow DNA poly-

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gated secondary antibody and detected using diaminobenzidine sub-

strate. The sections were counterstained briefly with hematoxylin, and
images were obtained using a Leica microscope (Leica Microsystems).

Densitometric and statistical analysis. Enhanced chemiluminescence images of immunoblots were scanned using a flatbed scanner (Hewlett Packard, Palo Alto, CA) and quantified using Zero D-Scan (Scanalytics, Fairfax, VA). Data are presented as means ± SE. The statistical differences were analyzed by ANOVA for comparison among multiple groups or Student’s t-test for comparison between two groups. P ≥ 0.05 was considered not statistically significant different.

RESULTS

Activation of IKK/NF-κB pathway in liver following trauma and hemorrhage. Critical illnesses and injuries, including hemorrhagic shock, can initiate acute inflammatory responses characterized by increased production of inflammatory factors and activation of inflammatory pathways such as the IKK/NF-κB pathway (4, 49). Activation of the IKK complex depends on the phosphorylation sites within the activation loop of the kinase (15). To determine whether IKK kinases are activated in liver following trauma and hemorrhage, phosphorylation of IKK kinases was examined with an antibody specific for Ser181-phosphorylated IKKβ and Ser180-phosphorylated IKKα. Increased phosphorylation/activation of IKKβ and IKKα was detected after TH90 compared with T90 (Fig. 1A). When measured, total IKKα and total IKKβ levels were not altered by trauma and hemorrhage (Fig. 1A) at the times that phosphorylated IKKβ and phosphorylated IKKα were induced.

NF-κB, a downstream target of the IKK complex, is an important transcription factor regulating expression of inflammatory factors (15). Activation of NF-κB in liver after trauma and hemorrhage was examined by gel-shift assay with a radiolabeled NF-κB consensus sequence probe, and DNA binding activity of NF-κB was significantly higher at TH90 than T90 (representative blot, Fig. 1B). These studies indicate an activation of the IKK/NF-κB pathway in response to hemorrhage. In additional experiments, there was a slight increase in DNA binding activity of NF-κB in trauma-only animals compared with normal (no trauma or hemorrhage) animals. This suggests a minor activation of this pathway by trauma alone and agrees with our previous work indicating a mild level of insulin resistance following trauma alone (28).

Time course of IKK and JNK activation. We have found that hepatic insulin resistance developed at TH15', as indicated by severely decreased insulin-induced Akt phosphorylation (Fig. 2) (24). In parallel experiments, the time course of IKKβ, JNK1, and phosphorylation of IRS1 at Ser312 in liver following trauma and hemorrhage was examined. Significantly elevated phosphorylated IKKβ, phosphorylated JNK1, and Ser312-phosphorylated IRS1 were observed at TH15' compared with trauma-alone groups, with levels continuing to increase through ≥90 min (Fig. 2). We demonstrated previously that the total levels of Akt, JNK, and IRS1 are not affected by trauma and hemorrhage (28, 52). The induction of IKKβ, JNK1, and Ser312 IRS1 phosphorylation presented here are not dependent

Fig. 2. Time course of Akt, JNK1, IKKβ, and Ser312 insulin receptor substrate 1 (IRS1) phosphorylation following trauma and hemorrhage. Rats were subjected to trauma and hemorrhage (TH) or trauma alone (T), and tissues were harvested at 15, 30, 60, and 90 min. A: Western blot analysis of liver protein extracts exposed to anti-Ser473-phosphorylated Akt (PS473-Akt), Thr181/Tyr183-phosphorylated JNK1/2 (P-JNK), Ser181-phosphorylated IKKβ/Ser180-phosphorylated IKKα, Ser312-phosphorylated IRS1 (PS312-IRS1), and total ERK antibodies. B: time course of Akt, JNK1, IKKβ, and Ser312 IRS1 phosphorylation after trauma and hemorrhage and trauma alone. Values are means ± SE (n = 6 for each group). *P < 0.05; **P < 0.01; ***P < 0.001 vs. T at the corresponding time point.
on the administration of insulin but are due to hemorrhage. This early activation of IKKβ, JNK1, and Ser112 IRS1 phosphorylation may allow them to play a role in the rapid development of insulin resistance in liver following trauma and hemorrhage.

Inhibition of IKK kinases resulted in improved hepatic insulin signaling. To directly investigate the role of IKKβ in hepatic insulin signaling following trauma and hemorrhage, rats were injected with Ad vectors expressing DN IKKβ (Ad-DN-IKKβ, 10^10 pfu) via the tail vein. Since IKKα, also a serine kinase, exists in the complex interacting with IRS1 (13), it may also contribute to IRS1 serine phosphorylation and participate in insulin resistance. We therefore included two other groups: a group injected with Ad-DN-IKKα (10^10 pfu) and a group injected with Ad-DN-IKKβ (5 × 10^9 pfu) + Ad-DN-IKKα (5 × 10^9 pfu). At 4 days postinjection, the animals were subjected to TH60° and injected with insulin or saline immediately before tissue was harvested. Expression of DN IKK kinases in liver in all rats was confirmed by Western blot analysis with total IKKα- or IKKβ-specific antibodies, which recognize endogenous IKK kinases and the DN mutants of the IKK kinases. The expression of endogenous IKKα or IKKβ was not affected by the control Ad vector Ad-nt-LacZ.

Fig. 3. Effects of IKK kinase inhibition on hepatic insulin signaling after trauma and hemorrhage. Rats were injected with adenovirus (Ad) vectors expressing dominant-negative (DN) mutant of IKKα [Ad-DN-IKKα; 10^10 plaque-forming units (pfu)] or IKKβ (Ad-DN-IKKβ; 10^10 pfu), control Ad vector (Ad-nt-LacZ; 10^10 pfu), or Ad-DN-IKKα (5 × 10^9 pfu) + Ad-DN-IKKβ (5 × 10^9 pfu) through the tail vein. At 4 days postinjection, animals were subjected to surgical trauma and hemorrhage, and tissues were harvested. A: Western blot analysis of liver lysates exposed to total IKKα-, total IKKβ-, and pan-actin-specific antibodies. B: immunohistochemistry was performed on frozen liver sections exposed to anti-hemagglutinin (HA) antibody. Magnification ×10. C: Western blot analysis of liver lysates exposed to Ser473-phosphorylated Akt and total Akt antibodies. D: fold change in Ser473-phosphorylated Akt. Values are means ± SE (n = 3–4 rats/group).

Fig. 4. Effects of IKK kinase inhibition on insulin-induced insulin receptor (IR) phosphorylation after trauma and hemorrhage. Rats were injected with Ad-DN-IKKα (10^10 pfu), Ad-DN-IKKβ (10^10 pfu), Ad-nt-LacZ (10^10 pfu), or Ad-DN-IKKα (5 × 10^9 pfu) + Ad-DN-IKKβ (5 × 10^9 pfu) through the tail vein. At 4 days postinjection, animals were subjected to surgical trauma and hemorrhage, and liver tissues were harvested. A: Western blot analysis of liver lysates exposed to Tyr972-phosphorylated IR (PY972-IR) and total IR antibodies. B: ratio of Tyr972-phosphorylated to total IR. Values are means ± SE (n = 3–4 rats/group).
(Fig. 3A) or by injection of the DN of the other IKK kinase, whereas expression of DN IKKα, IKKβ, or both, was achieved by their injection. Immunohistological examination indicated that a significant number, but not all, of the liver cells expressed the HA-tagged DN constructs (IKKβ, a representative liver section shown in Fig. 3B).

As expected, in the trauma-alone uninfected and the Ad-nt-LacZ-infected groups (T60'), insulin stimulated hepatic Ser473 Akt phosphorylation. However, induction of phosphorylated Akt was almost completely abolished after TH60' (Fig. 3, C and D). Expression of DN IKKβ in liver increased insulin-induced Akt phosphorylation following trauma and hemorrhage compared with the uninfected and the Ad-nt-LacZ control rats (Fig. 3, C and D). Significant improvements in insulin-induced Akt phosphorylation following trauma and hemorrhage were also detected following expression of DN IKKα. In addition, there was a significantly greater improvement in animals following administration of DN IKKα and IKKβ (Fig.

Fig. 5. Effects of JNK1 kinase inhibition on hepatic insulin signaling after trauma and hemorrhage. Rats were injected with Ad-DN-JNK1 (10^10 pfu) or Ad-nt-LacZ (10^10 pfu) through the tail vein. At 4 days postinjection, animals were subjected to surgical trauma and hemorrhage for 60 min, and liver tissues were harvested. A–C: Western blot analysis of liver lysates exposed to Thr181/Tyr183-phosphorylated JNK1/2-, total JNK1-, and pan-actin-specific antibodies. D and E: Western blot analysis of liver lysates exposed to Ser473-phosphorylated Akt and total Akt antibodies. F and G: Western blot analysis of liver lysates exposed to Tyr972-phosphorylated IR and total IR antibodies. Values in B, C, E, and G are means ± SE (n = 3–4 rats/group).
3, C and D). These results suggest that IKKα and IKKβ contribute to hemorrhage-induced defects in insulin signaling. However, for all groups, insulin-induced Akt phosphorylation was still lower following trauma and hemorrhage than following trauma alone, indicating only a partial recovery of insulin signaling by Ad-vector-mediated expression of DN mutant IKK kinases.

Next, insulin signaling upstream of Akt was examined. Tyr972 of the IR serves as binding site for IRS1 (54). Insulin-induced Tyr972 IR phosphorylation was impaired following trauma and hemorrhage in the uninfected and Ad-nt-LacZ control rats (Fig. 4A). Expression of DN IKKβ, DN IKKα, or both, resulted in increased Tyr972 IR phosphorylation in liver compared with uninfected and Ad-nt-LacZ control groups (Fig. 4). These results suggest that hemorrhage-induced defects in hepatic insulin signaling occur at the level of the IR, and IKK kinases may inhibit insulin signaling through decreasing IR tyrosine phosphorylation upstream of phosphatidylinositol 3-kinase/Akt signaling (Fig. 3).

Inhibition of JNK1 improved hepatic insulin signaling. To directly investigate the role of JNK1 kinase in the acute development of hemorrhage-induced hepatic insulin resistance, rats were injected with Ad vector expressing DN JNK1. At 4 days postinjection, the animals were subjected to surgical trauma and hemorrhage. Expression of DN-JNK1 was determined using a total JNK1-specific antibody. Total JNK1 increased consistently and significantly following administration of Ad-DN-JNK1 (Fig. 5, A and B). In the same animals, JNK1 phosphorylation was decreased by 50% following administration of Ad-DN-JNK1 (Fig. 5, A and C). Inhibition of JNK1 by Ad-DN-JNK1 also resulted in increased insulin-induced Akt phosphorylation (Fig. 5, D and E), as well as Tyr972 phosphorylation of IR after trauma and hemorrhage (Fig. 5, F and G), indicating a potential role of JNK1 kinase in hemorrhage-induced insulin resistance.

Inhibition of IKKα, IKKβ, or JNK1 results in decreased IRS1 Ser312 phosphorylation. JNK1 and IKKβ have been shown to associate with IRS1 and phosphorylate IRS1 at Ser312 in vitro (1, 13), which impairs IR (18) or IRS1 tyrosine phosphorylation (2). Inhibition of JNK1 and IKK kinases may act in parallel with JNK1, allowing for an additive effect when both are inhibited.

Cross talk between IKK and JNK kinases. To determine the cross talk between IKK and JNK kinases, effects of IKK or JNK inhibition on activation of the other kinase were examined. JNK1 inhibition did not alter the phosphorylation of IKKα or IKKβ (Fig. 8A), whereas expression of DN IKKα in liver resulted in a significant decrease in JNK1 phosphorylation/activation after trauma and hemorrhage (Fig. 8B). Unlike DN IKKα, there is no significant decrease of JNK phosphorylation after expression of DN IKKβ (data not shown). This suggests that IKKα may act upstream of JNK kinase following trauma and hemorrhage.

DISCUSSION

Critical illness diabetes, as evidenced by elevated blood glucose, is common in patients with injuries or critical illness due to increased hepatic glucose production and/or impaired peripheral glucose uptake, independent of previous diabetic status (29, 44, 46). The development of critical illness diabetes and its hyperglycemia in patients after acute injuries is of clinical concern because of the occurrence of adverse outcomes (6, 14, 22, 53). However, deleterious effects can also occur if the hyperglycemia is treated too aggressively by insulin, with increased hypoglycemic incidents (12). To delineate the mech-
anisms underlying the acute onset of insulin resistance after injuries, we have established an experimental rat model of critical illness diabetes. Our previous studies demonstrate a rapid development of insulin resistance in liver (23, 24, 27, 52). In the present studies, the rapid activation of JNK and another signaling pathway, the IKK/NF-κB pathway, was established. The causative roles of the IKK and JNK kinases in the acute development of insulin resistance were determined by direct inhibition of the IKK and JNK1 kinases with the Ad-mediated expression of DN mutants of IKKs and JNK1 in liver.

The response to injury/hemorrhage involves a complex coordination of the immune, cardiovascular, endocrine, and nervous systems (20), resulting in increased cellular stresses. These include (but are not limited to) oxidative stress and increased production of proinflammatory cytokines, which can then induce the activation of the JNK and IKK/NF-κB pathways (31). In the present studies, the time courses of IKK/NF-κB and JNK activation were examined and found to increase as early as 15 min and continued to increase in liver through the 90-min hemorrhage period. Thus activation of the JNK and IKK/NF-κB pathways may represent early and rapid signaling events in liver following injury/hemorrhage. Activation of IKK/NF-κB and JNK continued to increase and was maintained throughout the 90-min hemorrhage period, as was the defect in insulin signaling. In separate studies (57), we found that hemorrhage induces a rapid increase of reactive oxygen species (ROS) in liver, and inhibition of the increase of
ROS significantly decreased the acute development of hepatic insulin resistance. The data to date imply that the rapid activation of JNK and IKKs may be caused by the increase of ROS levels during hemorrhage.

Numerous studies have indicated the important role of IKKβ and JNK1 kinases in chronic insulin resistance in response to high-fat diet or obesity (5, 7, 13, 16, 35). However, the role of acute activation of IKK and JNK kinases in the rapid development of insulin resistance is not known. Unlike high-fat diet-induced obesity or type 2 diabetes, which are chronic problems that take many weeks, months, years, or decades to develop, the development of critical illness diabetes occurs very rapidly after an injury or infection. There is no a priori reason to believe that critical illness diabetes will have the same or different mechanisms of development. Since the mechanisms of development are unknown and critical illness diabetes is clearly an important clinical problem, the mechanisms need to be explored. In the present studies, DN IKKβ, IKKα, or JNK1 expression significantly improved insulin signaling, suggesting that the early activation of the IKK and JNK kinases contributes to the rapid onset of insulin resistance in critical illness diabetes. Injection of Ad-DN-IKKβ + Ad-DN-JNK1 resulted in additive effects on insulin sensitivity, further confirming that both contribute to the development of insulin resistance and may act in parallel. A partial causative factor in the acute development of hepatic insulin resistance within critical illness diabetes may be the activation of the IKK and JNK signaling pathway within minutes of hemorrhage.

However, defects in hepatic insulin signaling were only partially recovered by Ad-mediated expression of DN IKKs or DN JNK1, or both. The immunohistochemical staining of liver sections indicated that a large number of liver cells, but certainly not all hepatocytes, expressed DN proteins delivered by the Ad vectors. Since the DN protein works intracellularly, only those cells infected and expressing sufficient quantities of the DN protein will be affected. This may, in part, account for the partial blockade of the development of insulin resistance (partial increase of insulin signaling) after DN IKK and DN JNK1 expression. In addition, the Ad vector itself can initiate host inflammatory responses (19) and activate the JNK and IKK pathways (30, 33, 42), which may inhibit the activity of the DN IKK and JNK1 kinases and lower their effectiveness in blocking the development of insulin resistance in liver. Moreover, critical illness diabetes is complex, and we assume that there are multiple other causative factors and, therefore, potentially other signaling pathways, in addition to the IKK and JNK kinases, such as mammalian target of rapamycin and p38, which are likely involved in inducing the full insulin-resistant state. These other contributors remain to be determined in future studies on the development of acute insulin resistance in critical illness diabetes.

Among the three major subunits of the IKK complex, IKKβ is most clearly involved in the development of the chronic insulin-resistant state (5, 7, 13), with Nemo also necessary for TNFα-induced insulin resistance (34), whereas the role of IKKα in insulin resistance has not been as well studied. IKKα and IKKβ are catalytic subunits of the IKK complex and share extensive structural homology (36). IKKα and IKKβ contribute to inhibitory-κB phosphorylation and activation of NF-κB, and their similar abilities suggest that their functions are likely to be redundant and overlapping (36). In addition to forming heterodimers with IKKβ, IKKα can also form a homodimer, which may play a distinct role (36). Our data indicate an additive effect of expression of both DN constructs (DN IKKβ and DN IKKα), suggesting that they may also have separate actions in the development of acute insulin resistance in our animal model. Since IKKα exists in a complex that interacts with IRS1 (13), IKKα may also participate in...
inhibiting insulin signaling. Inhibition of IKKα by Ad-mediated expression of a DN mutant was found to improve hepatic insulin sensitivity in our model of critical illness diabetes. Thus, unlike chronic forms of insulin resistance, IKKα also likely contributes to acute insulin resistance that develops in critical illness diabetes.

There was an additive effect of expression of the DN forms of IKKβ and JNK1, suggesting that they act separately, potentially in parallel pathways, to cause acute insulin resistance. However, unlike IKKβ, no additive effect was detected following expression of DN IKKα and JNK1, suggesting that IKKα may act in series with JNK kinase in inhibiting hepatic insulin signaling in our model of critical illness diabetes. Further experiments demonstrated that inhibition of JNK kinase had no effect on IKKα or IKKβ activation, whereas expression of DN IKKα in liver resulted in decreased JNK1 activation. Together, these data suggest that IKKα acts upstream of JNK kinase in inducing hepatic insulin resistance. Thus, after activation of IKKα, IKKα can activate JNK kinase. In combination with other possible activators of JNK kinase and activation of a separate pathway containing activated IKKβ, there is a significant reduction of insulin signaling in our experimental model of critical illness diabetes.

Several studies have demonstrated cross talk between the IKK and JNK pathways, and IKK can act as a positive or negative regulator of JNK activation, depending on the type of stress (25, 40, 51). Phosphorylation of IKK is induced by a series of serine kinases, including MEKK1, MKK4, and MKK7. Thus, IKKα, a serine kinase, could phosphorylate/activate an intermediate protein that is important for JNK activation. Another possibility is that alterations in the activity of IKKα, an important kinase for canonical and noncanonical NF-κB activation, could result in altered expression of a number of genes, such as PKC, leading to decreased activation of the insulin signaling pathway (32). Thus, activation of serine kinases in the acute development of critical illness diabetes could result in decreased phosphorylation of the IR, leading to hemorrhage-induced hepatic insulin resistance. This possibility needs to be studied further, but no candidate serines of the IR have been identified.

In summary, we demonstrate that activation of the JNK and IKK/NF-κB pathways occurs early in the development of critical illness diabetes. The JNK1 and IKK kinases contribute to the acute development of critical illness diabetes-associated hepatic insulin resistance. Intensive insulin therapy has proven to be beneficial (47). However, there is some debate as to blood glucose target and, therefore, the intensity of insulin treatment that is most beneficial (11, 12, 21, 48). Our current studies suggest IKKs or JNK inhibitors as potential, novel therapeutic agents for treating or preventing the development of insulin resistance and hyperglycemia in critically ill patients.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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