Activation of Hepatic Inflammatory Pathways by Catecholamines Is Associated With Hepatic Insulin Resistance in Male Ischemic Stroke Rats

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Patients who experience acute ischemic stroke may develop hyperglycemia, even in the absence of diabetes. In the current study we determined the effects of acute stroke on hepatic insulin signaling, TNF-α expression, endoplasmic reticulum (ER) stress, the activities of c-Jun N-terminal kinase (JNK), inhibitor κB kinase β (IKK-β), and nuclear factor-κB (NF-κB) pathways. Rats with cerebral ischemia developed higher blood glucose, and insulin levels, and insulin resistance index, as well as hepatic gluconeogenic enzyme expression compared with the sham-treated group. The hepatic TNF-α mRNA and protein levels were elevated in stroke rats in association with increased ER stress, phosphorylation of JNK1/2 and IKK-β proteins, IκB/NF-κB signaling, and phosphorylation of insulin receptor-1 (IRS-1) at serine residue. The basal and insulin-stimulated tyrosine phosphorylation of IRS-1 and AKT proteins was reduced. In addition, acute stroke increased circulating catecholamines in association with hepatic adrenergic signaling activation. After administration of a nonselective β-adrenergic receptor blocker (propranolol) before induction of cerebral ischemic injury, hepatic adrenergic transduction, TNF-α expression, ER stress, and the activation of the JNK1/2, IKK-β, and NF-κB pathways, and serine phosphorylation of IRS-1 were all attenuated. In contrast, the phosphorylated IRS-1 at tyrosine site and AKT levels were partially restored with improved poststroke hyperglycemia and insulin resistance index. These results suggest that acute ischemic stroke can activate proinflammatory pathways in the liver by the catecholamines and is associated with the development of hepatic insulin resistance. **(*Endocrinology* 155: 1235–1246, 2014)**

A high proportion of patients with acute stress, such as stroke, may develop hyperglycemia, even in the absence of a preexisting diagnosis of diabetes (1–3). Human and animal studies suggest that stress-induced hyperglycemia is not a benign process and can be associated with a high risk of mortality after acute stroke (1–3). In contrast, lowering glucose can reduce ischemic brain damage (4, 5). In view of the effect of the blood glucose level on the prognosis of acute stroke, there is a need for further elucidation of the pathogenic mechanisms underlying poststroke glucose disturbances.

The liver is the main organ involved in endogenous glucose production, which is regulated by insulin through insulin receptor binding, and subsequent activation of the insulin receptor substrate (IRS)/phosphatidyl inositol 3-kinase/AKT pathway (6–8). Thus, if the liver becomes resistant to insulin signaling, the unsuppressed hepatic glucose output may become a significant contributor to

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Abbreviations: ATF, activation transcription factor; AUC, area under the curve; CREB, cAMP-responsive element binding protein; C/EBP, CCAAT/enhancer binding protein; CHOP, C/EBP homology protein; eIF2α, eukaryotic initiation factor 2 α; ER, endoplasmic reticulum; HOMA, homeostasis model assessment; HOMA-IR, HOMA insulin resistance; IκB-α, inhibitor κB-α; IKK-β, inhibitor κB kinase β; IRS, insulin receptor substrate; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor-κB; PKA, protein kinase A; PERK, protein kinase R-like ER kinase.

fasting hyperglycemia and postprandial hyperglycemia, as is observed in patients with diabetes or glucose intolerance (9, 10). In obese animals and humans, the liver has been shown to be accompanied with increased proinflammatory cytokine expression, such as TNF-α, which can activate intracellular c-Jun N-terminal kinase (JNK) and inhibitor κB kinase β (IKK-β), and phosphorylate insulin receptor substrate 1 (IRS-1) at the serine residue (11, 12), with impairment of hepatic insulin signaling (13, 14). Further, the activated IKK-β can also phosphorylate inhibitor κB-α (IκB-α), a protein bound to the nuclear factor-κB (NF-κB) complex, and thus cause its degradation and separation from NF-κB (15). This, in turn, allows translocation of NF-κB into the nucleus, induces the expression of a number of other inflammatory proteins (eg, TNF-α), and reinforces the deleterious effects of the inflammatory cascade on hepatic glucose metabolism. In several experimental acute illness models, such as trauma and burn injury, a relationship between hepatic inflammation and insulin resistance has been reported (16–18). However, such roles of hepatic inflammatory response in the development of poststroke glucose metabolism disorder are less studied before.

Using a stroke animal model, we have recently demonstrated that acute cerebral ischemic injury can induce hyperglycemia during the early phase of cerebral stroke (19, 20). Because previous studies have demonstrated that brain injury can induce an acute inflammatory response in the liver, including increased JNK activity and TNF-α levels (21, 22), we hypothesized that the hepatic TNF-α level, and JNK and IKK-β/NF-κB signaling might be up-regulated in response to cerebral ischemia, and become one of the events determining poststroke hyperglycemia. In the present study we determined whether or not acute cerebral ischemia induces insulin resistance in the liver and is associated with the hepatic inflammatory response. In addition, catecholamine has been shown to be activated in an ischemic stroke animal model (23, 24), and reported to stimulate the expression of proinflammatory cytokines in the liver (25). Therefore, the present study also examined whether or not the administration of a nonselective β-adrenergic receptor blocker (propanolol) could alter hepatic inflammation following ischemic stroke and the poststroke insulin signaling in the liver.

Materials and Methods

Animals and induction of cerebral ischemia

The Animal Experimental Committee of Taichung Veterans General Hospital approved the protocol of this animal study. Adult male Sprague Dawley rats (300–350 g) were anesthetized with chloral hydrate (400 mg/kg ip). The body temperature of each rat was maintained at 37.0 ± 0.5°C with a heating pad. Focal ischemic infarcts in the right lateral cerebral cortex were produced by clamping the 2 common carotid arteries and the right middle cerebral artery, as described previously (19, 20). In animals undergoing sham operations, all surgical procedures were the same as above, but no arterial occlusion was performed.

Quantification of cerebral infarction

The brains were quickly removed and chilled in cold PBS for 5 minutes, and 2-mm coronal slices were cut using a tissue slicer. The slices were immersed in a PBS solution containing 2% triphenyltetrazolium chloride at 37°C for 30 minutes, after which sections were fixed in 10% phosphate-buffered formalin for 45 minutes.

Experimental design of the study

All rats were randomly allocated into 2 study groups. In the first group, animals were divided into sham-operated and ischemia subgroups (n = 8/subgroup). In the second group, animals were divided into 4 subgroups: sham with normal saline; sham with insulin; ischemia with normal saline; and ischemia with insulin (n = 8/subgroup), in which ip insulin (10 U/kg) or normal saline injection was administrated 15 minutes before the animals were humanely destroyed.

Blood, liver, and adipose tissue preparation

On day 1 after the ischemic brain injury, the experimental and sham-operated rats were humanely destroyed in the morning without particularly prior food withdrawal at night (basal status). After anesthesia with chloral hydrate (400 mg/kg ip), intraarterial catheterization of the left femoral artery was performed for blood sampling. Blood was immediately centrifuged, and plasma samples were stored at −70°C until analysis. In addition, the liver and epididymal adipose were rapidly dissected, weighed, and stored in liquid nitrogen until analysis. In some experiments, to determine the effects of cerebral ischemia on hepatic insulin signaling, food was first withdrawn for 12 hours after which insulin (10 U/kg) was administrated ip 15 minutes prior to euthanasia on day 1 after ischemic brain injury.

Determination of glucose tolerance and insulin resistance

Before and 1 day after inducing ischemic brain injury, blood was sampled for glucose analysis prior to glucose loading and 30, 60, and 120 minutes after injecting glucose solution (2 g/kg, ip). All animals were fasted before glucose injection (n = 6/subgroup), and blood glucose was monitored in the tail veins using a hand-held Accucheck glucometer (Roche Diagnostics). The total area under the curve (AUC) for glucose during the ip glucose tolerance test ($AUC_{2h\ glucose}$) was calculated using the trapezoidal (trapezium) rule. Insulin resistance was determined in fasting rats according to the homeostasis model assessment (HOMA), as described by Matthews et al (26). The HOMA insulin resistance (HOMA-IR) index was calculated as follows: [fasting insulin (μIU/mL) × fasting glycemia (μmoL/L)]/22.5. Blood insulin levels were measured using a rat/mouse ELISA kit (Linco Research, Inc) according to the manufacturer's instructions.

Analyses of cytokine, alanine aminotransferase, and catecholamines

The liver was thoroughly homogenized in tissue protein extraction reagents (T-PER; Pierce Biotechnology), and the supernatant fluids after centrifugation were collected. The hepatic and blood samples were assayed for TNF-α and monocyte chemoattractant protein 1 (MCP-1) using a rat inflammation kit (BD Bioscience). Serum alanine aminotransferase levels were measured on an autoanalyzer in our biochemistry department. Blood norepinephrine and epinephrine were measured with commercial ELISA kits according to the manufacturer's descriptions (Labor Diagnostika Nord GmbH & Co).

Administration of propranolol

In some experiments, propranolol (2 mg/kg) was injected ip 3 hours before the induction of cerebral ischemia. Thereafter, the blood and hepatic tissues were collected as described previously.

RNA preparation and gene expression analysis

Total mRNA was extracted from frozen hepatic tissue samples using TriZol agents (Life Technologies, Inc) and the mRNA concentrations were determined by UV light absorbance at 260 nm. The expression of hepatic proinflammatory cytokine mRNA was determined by semiquantitative RT-PCR analysis normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Briefly, 1–5 μg of total RNA was transcribed using reverse transcriptase and poly $(dT)_{12-18}$ primer, after which a PCR was conducted in a DNA Thermal Cycler 480 (PerkinElmer Co.). The primer sets used in this study were as follows: forward, 5′-CCCTCACACTCAGATCATCTTCTCAA; and reverse, 5′-TCTAAGTACTTGGGCAGGTTGACCTC for TNF-α; forward, 5′-TGCAGGTCTCTGTCACGCTTC; and reverse, 5′-TTCTCCAGCCGACTCATTGG for MCP-1; and forward, 5′-ACCACAGTCCATGCCATCAC; and reverse, 5′-TCCACCACCCTGTTGCTGTA for GAPDH. The target gene and GAPDH bands were measured by densitometry using Image Quant Analysis software.

Western blot analyses

Total proteins were extracted from liver and adipose tissues (100 mg) using tissue protein extraction reagents (T-PER, Pierce Biotechnology). Protein extracts were resolved by SDS-PAGE and transferred onto a blotting membrane. To determine the effect of acute cerebral ischemia on hepatic gluconeogenic enzyme expression, 100 μg homogenates were subjected to electrophoresis, and incubated with glucose-6-phosphatase and

Table 1. Serial Change of Metabolic Characteristics in Sham-Operated and Cerebral Ischemia Rats

	Baseline (n = 6–8)	Day 1 Without propranolol (n = 6–8)	Day 1 With propranolol (n = 6–8)
Body weight (g)			
Sham	305.8 ± 3.8	303.2 ± 3.9	304.5 ± 3.7
Ischemia	304.3 ± 3.8	275.2 ± 5.9 [a]	277.4 ± 5.8
Liver weight (g)			
Sham	9.5 ± 0.5	9.3 ± 0.3	9.3 ± 0.4
Ischemia	9.5 ± 0.4	9.3 ± 0.4	9.2 ± 0.5
Infarct volume (%)			
Sham			
Ischemia		28.0 ± 3.4 [a]	27.8 ± 3.4 [a]
Blood norepinephrine (ng/mL)			
Sham	4.4 ± 0.2	8.1 ± 0.4	6.4 ± 0.4
Ischemia	4.3 ± 0.4	12.2 ± 0.3 [a]	6.8 ± 0.4 [b]
Blood epinephrine (ng/mL)			
Sham	6.2 ± 0.2	6.3 ± 0.4	6.9 ± 0.4
Ischemia	6.3 ± 0.4	11.2 ± 0.3 [a]	7.2 ± 0.4 [b]
Fasting glucose (mM)			
Sham	4.4 ± 0.4	4.5 ± 0.4	4.4 ± 0.4
Ischemia	4.5 ± 0.3	5.9 ± 0.3 [a]	5.0 ± 0.4 [b]
Fasting insulin (μIU/mL)			
Sham	7.7 ± 0.2	7.7 ± 0.2	7.6 ± 0.2
Ischemia	7.7 ± 0.3	15.3 ± 3.8 [a]	7.8 ± 2.2 [b]
HOMA-IR			
Sham	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2
Ischemia	1.5 ± 0.3	4.0 ± 1.2 [a]	1.7 ± 0.9 [b]
Total AUC during IPGT (mg/dL × hour)			
Sham	320 ± 25	326 ± 25	315 ± 22
Ischemia	315 ± 22	495 ± 32 [a]	422 ± 32 [b]
Blood ALT (IU/mL)			
Sham	43 ± 13	45 ± 14	42 ± 15
Ischemia	42 ± 14	66 ± 16 [a]	45 ± 16 [b]

Data are given as means ± SEM. [a], $P < .05$ sham vs ischemia; [b], $P < .05$ ischemia with saline vs ischemia with propranolol. IPGT, ip glucose tolerance test.

phosphoenolpyruvate carboxykinase antibodies (Santa Cruz Biotechnology, Inc.). To determine the effect of acute stroke on hepatic insulin signaling, the hepatic homogenates (100 μg protein) were immunoblotted with antibodies to insulin receptor (IR), phosphorylated IR (Tyr-1150/1151), IRS-1, phosphorylated IRS-1 (Tyr-896), AKT, and phosphorylated AKT (Ser-473; Santa Cruz Biotechnology, Inc). In addition, to examine whether or not cerebral ischemia induces hepatic inflammation, liver homogenates (100 μg protein) were immunoblotted with anti-CD68, anti-TNF-α, JNK (R&D Systems), phosphorylated JNK (Thr-183/Tyr-185; BD Biosciences), IκB-α, and total and phosphorylated IKK-β (Ser-176/180) (Sigma-Aldrich). To examine whether or not cerebral ischemia induces hepatic endoplasmic reticulum (ER) stress, liver homogenates (100 μg protein) were immunoblotted with antibodies against protein kinase R-like ER kinase (PERK), eukaryotic initiation factor 2 α (eIF2α), activation transcription factor 4 (ATF4), ATF6, CCAAT/enhancer binding protein (C/EBP) homology protein (CHOP), phosphorylated PERK(p-PERK; Santa Cruz Biotechnology, Inc), and phosphorylated eIF2α (p-eIF2α; Cell Signaling Technology). Finally, the adrenergic signaling transduction mediators, including cAMP-responsive element binding protein (CREB), phosphorylated CREB (p-CREB), and the other effectors, ERK, phosphorylated ERK (p-ERK1/2), p38 MAPK, and phosphorylated p38 (p-p38; Santa Cruz Biotechnology, Inc), were also determined. Blotted membranes were then stained with antirabbit or antigoat IgG secondary antibodies labeled with horseradish peroxidase (Amersham Co). Specific protein bands were visualized by enhanced chemiluminescence (Amersham Co). All measured protein levels were quantified by densitometry, and the percentage change relative to sham-operated rats was determined after normalization by GAPDH with diluted anti-GAPDH antibodies (1:3000; Santa Cruz Biotechnology, Inc).

Protein kinase A (PKA) activity assay

PKA activity was measured using the PKA kinase activity kit according to the manufacturer's descriptions (Enzo Life Sciences, International Inc).

Nuclear extraction and EMSA

Nuclear proteins were extracted from liver tissues, and EMSA was conducted, as described previously (27). The oligonucleotides specific for nuclear factor-κB (NF-κB) (5′-AGTTGAGGGGACTTTCCCAGGC) were synthesized and labeled with biotin. Nuclear extract (5 μg) was used for EMSA. The binding reaction mixture included 1 μg of poly(dI-dC), 0.1 μg of poly *l*-lysine and 100 fmol of biotin-labeled DNA probe in a 20-μL binding buffer (10 mM HEPES, pH 7.6; 50 mM NaCl; 0.5 mM $MgCl_2$; 0.5 mM EDTA; 1 mM dithiothreitol; and 5% glycerol). The DNA-protein complex was analyzed on 6% native polyacrylamide gels.

Flow cytometric analysis

Red blood cells in the collected blood was first lysed in hypotonic buffer (0.05% NaCl) and then reversed tonic in hypertonic buffer

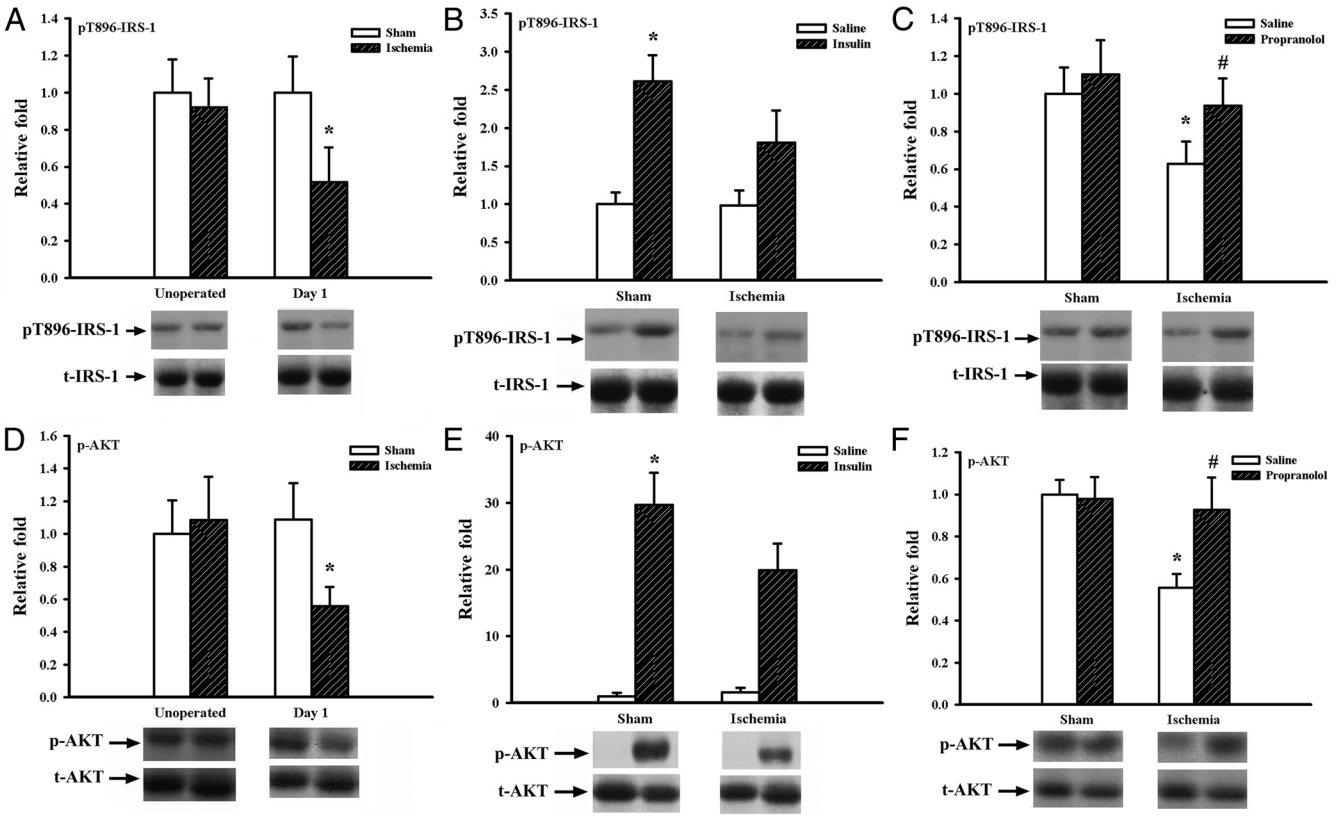

Figure 1. Representative expression of phosphorylated IRS-1 at tyrosine 896 residue (pT896-IRS-1), AKT (p-APK), and associated total proteins in liver 1 day after ischemic surgery and with and without preischemia propranolol administration. Data in each group (n = 6–8) were expressed as a fold change of control rats before surgery or sham-surgery rats without insulin or saline-treated sham-surgery rats and given as means ± SEM. *, $P < .05$ sham vs ischemia; #, $P < .05$ ischemia with saline vs ischemia with propranolol.

(1.75% NaCl and 2% NaCl). For the detection of inflammatory cells, the isolated cells were washed in PBS and stained with monoclonal antibody against CD45 (BioLegend). Antibody-labeled cells were washed and fixed in PBS with 0.37% formaldehyde. Characterization of antibody-labeled cells was performed on a BD FACScalibur flow cytometer. The data obtained were then analyzed using Cell Quest software (BD Biosciences).

Liver immunohistochemistry

For immunohistochemical examination, sections of formalin-fixed, paraffin-embedded hepatic tissue were deparaffinized and incubated with anti-CD68 and anti-TNF-α antibodies. Then sections were washed 3 times in TBST (20 mmol/L Tris, 137 mmol/L NaCl, 0.05% Tween 20, pH 7.5), and incubated with a biotinylated secondary antibody, followed by an avidin-biotin-peroxidase complex. The immunoreactive signal was developed by color deposition using diaminobenzidine as substrate. The average number of CD68- and TNF-α-positive immunoreactivity per observation was counted in 6 continuous sections.

Statistical analysis

All data are expressed as the mean ± SEM. Differences were determined using Student's unpaired *t* test. Comparisons among several groups were compared statistically by ANOVA, followed by the Bonferroni multiple comparison test. Results were considered significant at $P < .05$. All data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 6.0 for Windows; SPSS, Inc).

Results

Changes in glucose tolerance following acute cerebral ischemia

In comparison with sham-operated animals, rats subjected to cerebral ischemia exhibit brain infarctions after 24 hours, mainly in cortical areas, as previously reported (Table 1 and Supplemental Figure 1 published on The

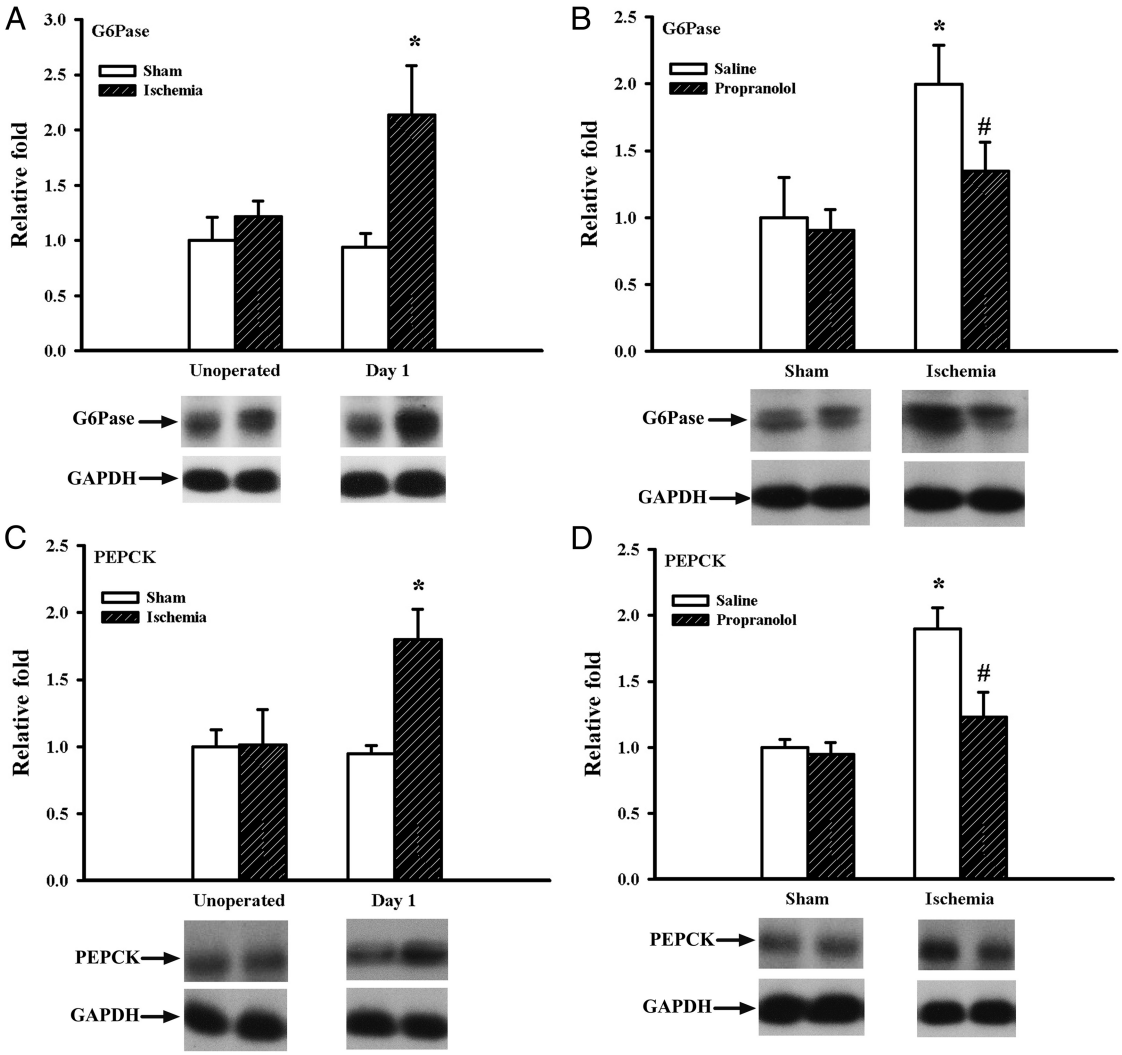

Figure 2. Representative expression of glucose-6-phosphatase (G6pase) and phosphoenolpyruvate carboxykinase (PEPCK) protein levels in liver 1 day after ischemic surgery and with and without preischemia propranolol administration. Data in each group (n = 6–8) were expressed as a fold change of control rats before surgery or saline-treated sham-surgery rats and given as means ± SEM. *, $P < .05$ sham vs ischemia; #, $P < .05$ ischemia with saline vs ischemia with propranolol.

Endocrine Society's Journals Online web site at http://endo.endojournals.org) (19, 20). The body and liver weights of the rats were significantly decreased on day 1 after the induction of cerebral ischemia compared with before surgery (Table 1). The rats with acute cerebral ischemia had higher blood insulin, glucose, and HOMA-IR levels compared with the sham-operated rats (Table 1). After the ip injection of glucose, the stroke rats had a higher postload glucose AUC compared with control rats (Table 1 and Supplemental Figure 2). In addition, serum hepatic enzyme concentrations were significantly elevated in stroke rats.

Altered insulin signaling and gluconeogenic enzyme expression in liver postischemic stroke

The basal expression of phosphorylation of IRS-1 on tyrosine and AKT on serine in the liver was significantly lower in the stroke rats than the sham group on day 1 after induction of cerebral ischemia, although there was no difference in total IRS-1 and AKT proteins between the cerebral ischemia and sham-operated animals (Figure 1). After the ip administration of insulin, cerebral ischemic animals also showed less stimulation of phosphorylation of IRS-1 at tyrosine, and AKT postischemic stroke (Figure 1). In addition, consistent with our previous study (19), there was also a less insulin-stimulated IRS-1 at tyrosine and AKT expression in adipose tissue of acute stroke rats (Supplemental Figure 3). The protein expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase was significantly higher in the rats subjected to cerebral ischemia than in the sham-operated animals (Figure 2). These findings suggest that hepatic insulin resis-

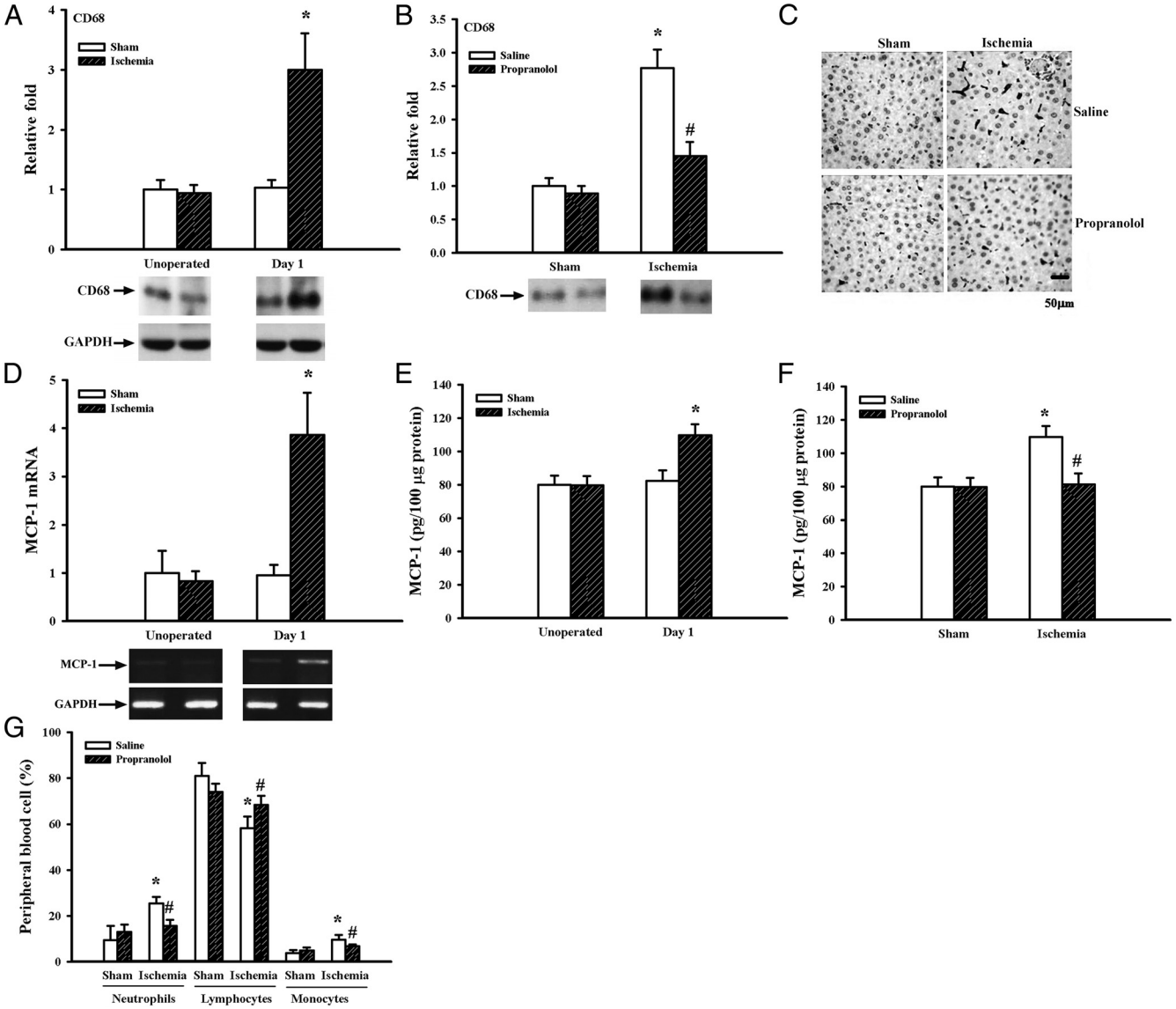

Figure 3. Peripheral blood cell fraction, and representative expression of MCP-1, CD 68, and immunohistochemical statining (×400; scale bar = 50 μm) in liver 1 day after ischemic surgery and with and without preischemia propranolol administration. Data in each group (n = 6–8) were expressed as a fold change of control rats before surgery or saline-treated sham surgery and given as means ± SEM. *, $P < .05$ sham vs ischemia; #, $P < .05$ ischemia with saline vs ischemia with propranolol.

tance developed after cerebral ischemia and was accompanied by increased gluconeogenic gene expression.

Change in the peripheral blood cells, CD68, and TNF-α expression in liver tissue postischemic stroke

In the peripheral blood, the fraction of lymphocytes was decreased in stroke rats on day 1 after brain ischemia, but monocyte and granulocyte numbers were increased (Figure 3). The hepatic levels of MCP-1 and CD68 (a macrophage marker) were increased in parallel with histochemical analysis of liver in CD 68-positive cell numbers (Figure 3). In addition, hepatic expression of TNF-α mRNA and protein levels and TNF-α-positive cells were also significantly elevated in the stroke rats on the first day following surgery (Figure 4), although the circulating TNF-α concentrations were not changed (data not shown).

Acute cerebral ischemia-induced activation of JNK and IKK-β/NF-κB pathways and ER stress

In parallel with increased hepatic TNF-α expression, the phosphorylated JNK-1/2 and IKK-β levels were significantly increased in the livers of stroke rats the first day after artery ligation with higher levels of phosphorylated ISR-1 at the serine residue (Figure 5). In addition, because IKK-β induces phosphorylation and degradation of IκB protein and subsequent activation of NF-κB, the phosphorylated IκB-α protein level and NF-κB activity were measured (28). In the stroke rats, phosphorylated IκB-α protein was increased in the liver, but total IκB-α levels were decreased (Figure 5). The NF-κB DNA binding activity in the rats with cerebral ischemia was increased by 1- to 2-fold compared with the sham-operated controls (Figure 5). In addition, several markers associated with ER stress, including PERK, eIF2α, CCAAT/enhancer binding protein (C/EBP) homology protein, ATF 4, and ATF 6, were also raised in the liver of acute stroke rats (Supplemental Figure 4).

Acute cerebral ischemia increased catecholamines and activated adrenergic signaling in liver

In the stroke rats, circulating norepinephrine and epinephrine levels were significantly elevated compared with

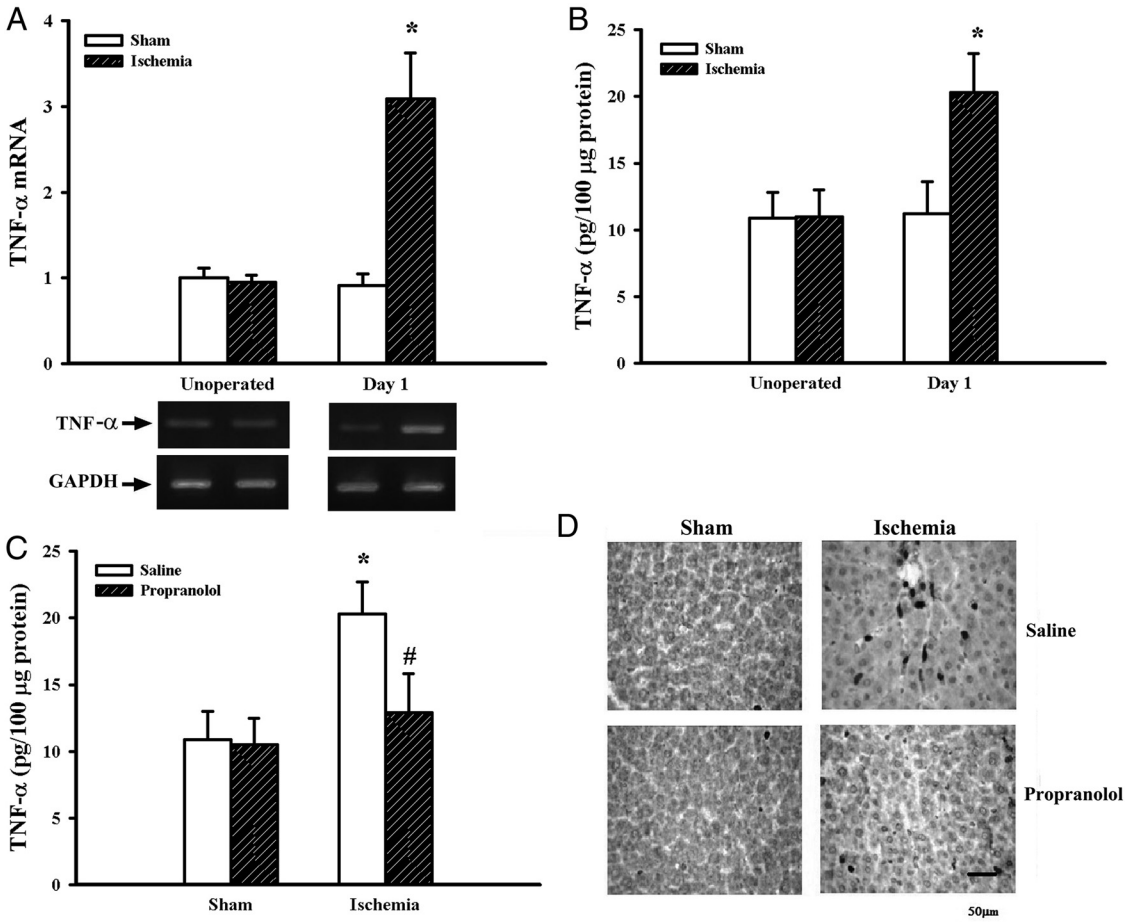

Figure 4. Representative expression of TNF-α and immunohistochemical staining (×400; scale bar = 50 μm) in liver 1 day after ischemic surgery and with and without preischemia propranolol administration. Data in each group (n = 6–8) were expressed as a fold change of control rats before surgery or saline-treated sham surgery and given as means ± SEM. *, $P < .05$ sham vs ischemia; #, $P < .05$ ischemia with saline vs ischemia with propranolol.

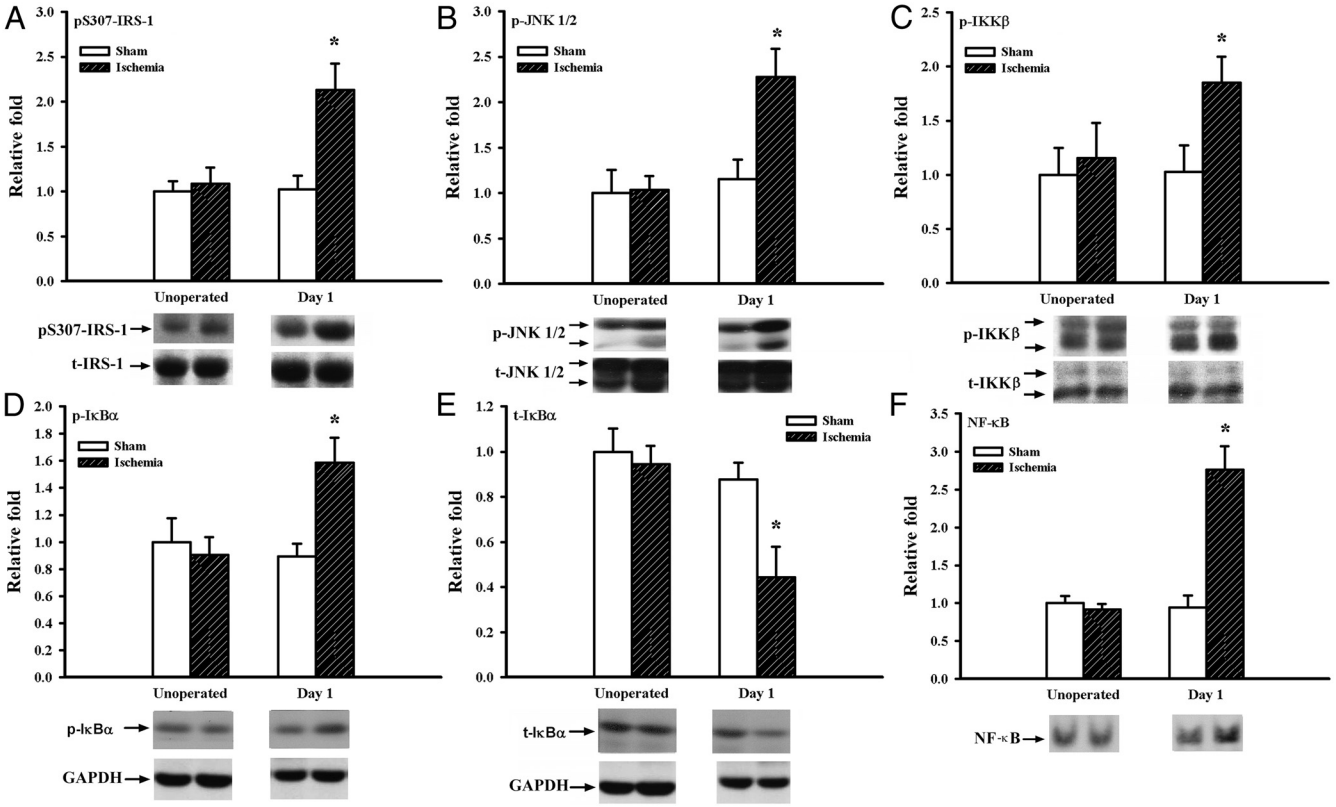

Figure 5. Representative expression of phosphorylated IRS-1 at serine 307 residue (pS307-IRS-1), JNK (p-JNK1/2), IKKβ (p-IKKβ), and associated total proteins, and IκB-α (p-IκB-α), total inhibitor κB-α (t-IκB-α), and NF-κB activity in liver 1 day after ischemic surgery. Data in each group (n = 6–8) were expressed as a fold change of control rats before surgery and given as means ± SEM. *, $P < .05$ sham vs ischemia.

sham controls (Table 1). Concurrently, the adrenergic signaling transduction pathway, including PKA activity, and phosphorylation of CREB, ERK1/2, and p38, was stimulated in the liver of rats with cerebral ischemia (Figure 6). However, the expression of β-adrenergic receptor mRNA showed no difference between ischemic and sham control rats (data not shown).

Propranolol effect on changes in adrenergic signaling, TNF-α, CD 68, JNK, and IKK-β/NF-κB pathways, ER stress, insulin signaling, and gluconeogeneic enzyme expression in the liver after acute cerebral ischemia

To determine a potential role of catecholamines in the increased expression of TNF-α and activation of intracellular JNK and IKK-β pathways in liver tissues following cerebral ischemic stroke, we administered propranolol before the induction of cerebral ischemia, and showed that the stroke-induced increase in hepatic TNF-α, MCP-1, and CD 68 levels were diminished (Figures 3 and 4) in association with decrease in peripheral blood monocytes, TNF-α-, and CD 68-positive cells of liver (Figures 3 and 4), but without an effect on the infarct volume (Table 1 and Supplemental Figure 1). In addition, the adrenergic signaling mediators, including PKA activity and phosphorylation of CREB, ERK1/2, and p38, were also decreased (Figure 6). Phosphorylated JNK 1/2, phosphorylated IKK-β, NF-κB DNA binding activity, and serine phosphorylation of IRS-1, as well as ER markers, were attenuated in stroke rats the first day after artery ligation (Figure 7 and Supplemental Figure 4). On the contrary, the phosphorylated IRS-1 at tyrosine site and AKT levels in liver and epididymal fat were partially restored (Figure 1 and Supplement Figure 3). Finally, fasting and postload glucose concentrations, and HOMA-IR (Table 1 and Supplemental Figure 2) and expression of hepatic gluconeogenic enzyme levels were lowered in stroke rats that received propranolol (Figure 2).

Discussion

In agreement with our previous report (19, 20), the current study using a rat stroke model showed that blood glucose, insulin, HOMA-IR, and postload glucose levels were significantly elevated following acute cerebral ischemia. The development of hyperglycemia after cerebral ischemia was accompanied by increased hepatic gluconeogenic enzyme levels. In addition, it was observed that the basal and insulin-stimulated expression of tyrosine phosphorylation

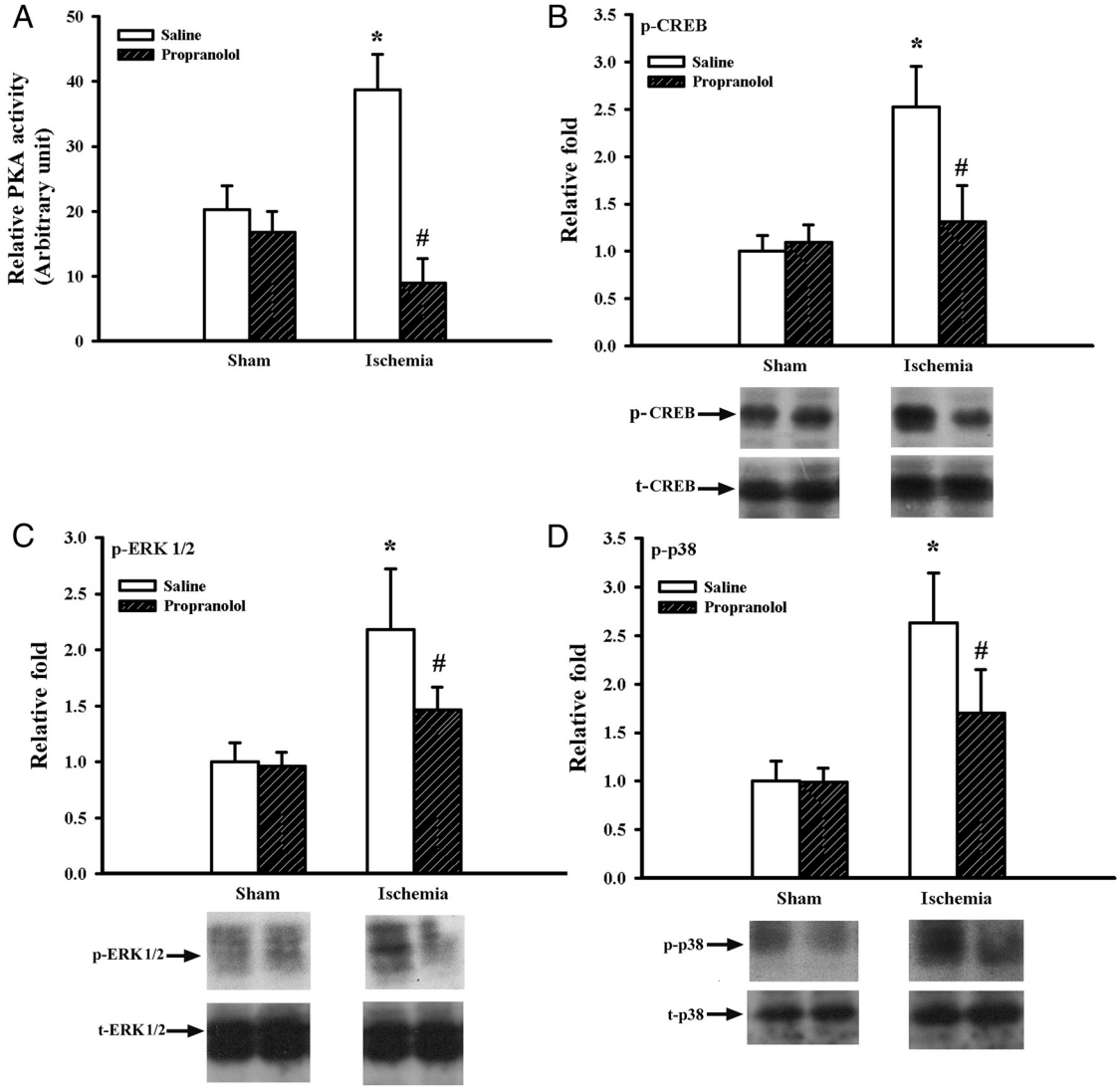

Figure 6. Representative expression of PKA activity, pCREB, p-ERK, and p-P38 in liver 1 day after ischemic surgery with and without preischemia propranolol administration. Data in each group (n = 6–8) were expressed as a fold change of saline-treated sham surgery and given as means ± SEM. *, $P < .05$ sham vs ischemia; #, $P < .05$ ischemia with saline vs ischemia with propranolol.

of IRS-1 and serine phosphorylation of AKT in the liver and epididymal fat were significantly lower in rats with acute cerebral ischemia than sham-operated animals on day 1 after ischemic injury.

Insulin mainly inhibits hepatic expression of rate-limiting gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase, and glucose-6-phosphatase through regulation of their transcription (29). Because hepatic glucose production is the main contributor to the plasma glucose level in the fasting state (6, 30), the higher basal blood glucose levels in stroke rats implicate an impaired insulin sensitivity in the liver after acute cerebral ischemia. In addition, this hepatic insulin resistance can also contribute to postload hyperglycemia observed in acute stroke rats due to unsuppressed hepatic glucose production (10).

Infiltrated leukocytes or activated resident macrophages in liver have been proposed to be an important source of peripheral proinflammatory cytokine production after brain injury (31, 32). In acute stroke rats, because peripheral blood monocytes and hepatic MCP-1 levels were increased, it is thought that circulating monocytes can spread into liver and become a source of increased hepatic TNF-α production after cerebral ischemia, although activation of inherent resident macrophages in the liver (eg, Kupffer cells) might be another possibility.

A large accumulation of data has documented the important role of IKK-β/NF-κB and JNK activation and ER stress in the pathogenesis of hepatic insulin resistance in obesity and diabetes (11, 12, 28, 33). In the current study, we demonstrated that the inflammatory pathway, as well as ER stress, was also activated locally in the liver after

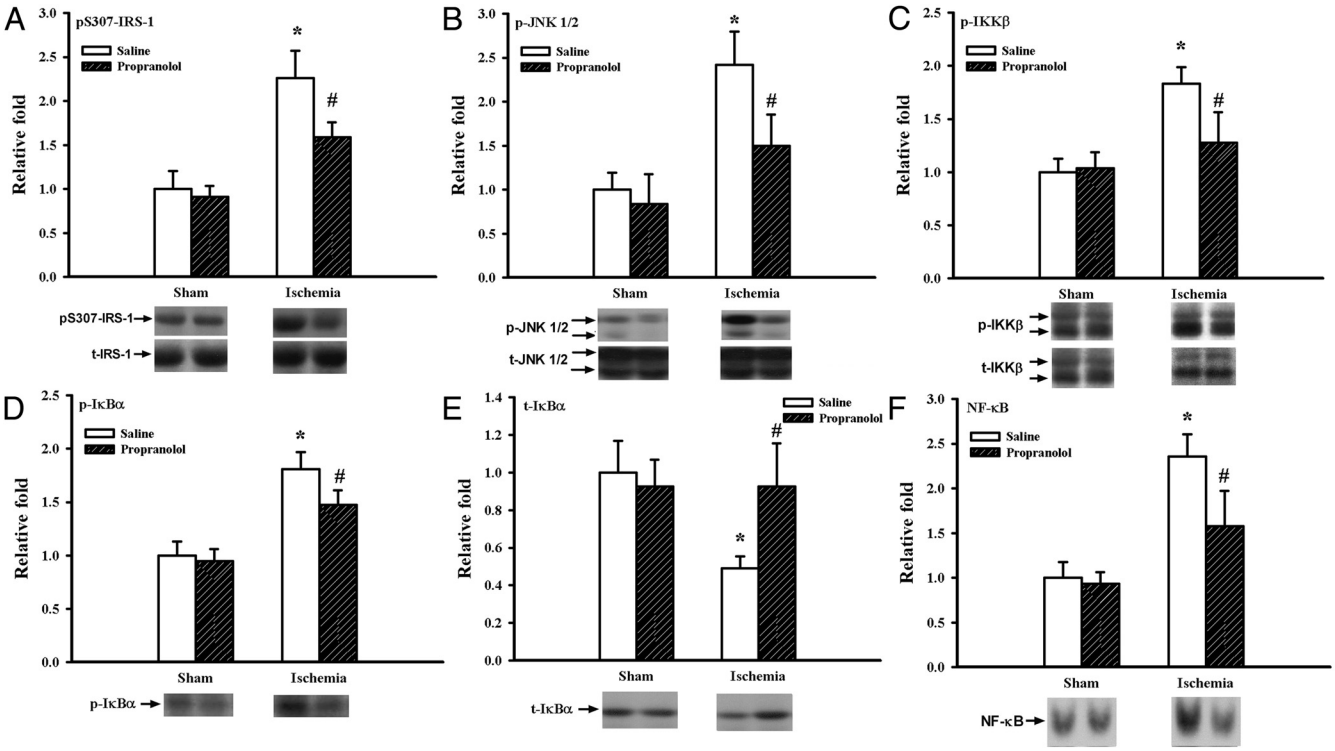

Figure 7. Representative expression of pS307-IRS-1, p-JNK1/2, p-IKKβ, and associated total proteins, and p-IκB-α, t-IκB-α, and NF-κB activity 1 day after ischemic surgery with and without preischemia propranolol administration. Data in each group (n = 6–8) were expressed as a fold change of saline treated sham surgery and given as means ± SEM. *, $P < .05$ sham vs ischemia; #, $P < .05$ ischemia with saline vs ischemia with propranolol.

acute cerebral ischemia, and associated with impaired hepatic insulin signaling, including decreased IRS-1 tyrosine and Akt phosphorylation, but increased serine phosphorylation of IRS-1, which, as a result, contributed to poststroke hyperglycemia. Our data mimicked the findings of previous studies in experimental hemorrhage and burn models (16–18) and would further extend the importance of local hepatic inflammation and ER in the pathogenesis of glucose metabolic abnormalities in acute illnesses.

Next, we examined whether catecholamines were involved in activation of hepatic inflammatory response after cerebral ischemia because they have been shown to stimulate expression of proinflammatory cytokines and ER stress in liver through β-adrenergic receptors (17, 25, 34). In acute stroke rats, circulating norepinephrine and epinephrine levels, which can be released from adrenal medulla as well as from sympathetic nerves, were increased. In addition, several signaling factors in the adrenergic pathway were stimulated in liver. Previous studies have shown that activation of ERK and p38 can mediate adrenergic agonist-induced proinflammatory cytokine production in microglia cells, macrophages, and cardiac fibroblasts (35–37). It is proposed that these 2 intracellular pathways may also be involved in hepatic inflammation in acute stroke rats. On the contrary, administration of propranolol partially suppressed increase in adrenergic signalings, hepatic inflammation, and ER stress in association with improved insulin transduction, and poststroke hyperglycemia. The reasons for reduced circulating catecholamines after propranolol treatment in stroke rats were not clear, and it has been reported that propranolol can inhibit norepinephrine release from postganglionic sympathetic neurons (38). Taken altogether, our experimental findings herein suggest a role of sympathoadrenal system overactivation in the development of impaired hepatic insulin signaling and thus glucose dysregulation in acute stroke. However, whether catecholamine may cause poststroke hyperglycemia by the other mechanisms, such as antagonizing hepatic insulin action directly, requires further clarification (39).

Because widespread inflammatory response in the liver can occur soon after ischemic brain injury (32), our observation may have clinical implication that preischemia adrenergic blockade can prevent stress effects on liver from catecholamine release after acute stroke. In fact, a recent human study demonstrates that poststroke hyperglycemia is improved in patients taking β-blocker treatment before stroke onset (40). However, despite this, the beneficial effects of β-blocker on stroke outcome show controversial results in the other human study (41). It is proposed that time point, duration, and dosage of β-adrenergic blockade administration can changes its effects on stroke-induced glu-

cose intolerance. In some previous experimental studies in sepsis and burn injury, adrenergic receptor antagonists are actually designed to be administrated after acute insults, which may more mimic clinical practice (42, 43). Thus, further investigation is needed before any conclusion can be made about clinical use of propranolol to improve stroke-associated glucose intolerance.

The present study had several limitations, and it is important to interpret the laboratory findings cautiously. First, only a cerebral ischemic model was used, and whether or not other stroke models, such as intracranial hemorrhage (1–3), have similar results is not known. Second, we did not directly address the exact causal relationship between the hepatic proinflammatory response and hyperglycemia in acute stroke rats. The increased hepatic TNF-α expression and IKK-β/NF-κB and JNK activity, as well as altered hepatic insulin signaling, might be possible secondary to poststroke hyperglycemia rather than the causes (44, 45). Third, the other factors, such as the counterregulatory hormones (eg, glucagon), may also contribute to the development of insulin resistance in acute stroke. In our recent report, glucagon is shown to be rapidly activated after acute stroke (20). Fourth, hemodynamic change after acute stoke may be another possible mechanism by catecholamines to induce hepatic inflammation and insulin resistance (46), although our laboratory had preliminarily found that expression of endothelial nitric oxide synthase, an important regulator of hepatic hemodynamics and insulin sensitivity (47), did not change in acute stroke rats (our unpublished observation). Finally, the beneficial effects of propranolol on the blood glucose level and hepatic inflammatory response were possibly due to the protection of neurons from ischemic injury, although this study did not show that a β-adrenergic blocker reduced the cerebral damage on day 1 after surgery.

In summary, the present study demonstrated that acute cerebral ischemia caused poststroke dysglycemia and impaired hepatic insulin signaling. In addition, the expression of hepatic TNF-α, activities of intracellular IKK-β, NF-κB, and JNK1/2, and ER stress were increased in association with catecholamine release and activated adrenergic signaling transduction. The administration of a β-adrenergic antagonist (propranolol) before induction of cerebral ischemia attenuated the poststroke hepatic inflammatory response and partially restored the insulin-signaling pathways in the liver. These results suggest that acute cerebral ischemia can activate inflammation pathways in the liver by the catecholamines and contribute to hepatic insulin resistance after a stroke.

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