Inducible nitric oxide synthase plays a role in LPS-induced hyperglycemia and insulin resistance

HIROKI SUGITA,1 MASAO KANEKI,1 ERIKO TOKUNAGA,1 MICHIKO SUGITA,1 CHIEKO KOIKE,2 SHINGO YASUHARA,1 RONALD G. TOMPKINS,3 AND J. A. JEEVENDRA MARTYN1

Departments of 1Anesthesia and Critical Care and 3Surgery, Harvard Medical School, and Anesthesia and Surgical Services, Massachusetts General Hospital and Shriners Hospital for Children, Boston, Massachusetts 02114; and 2Center for Developmental Biology, University of Texas Southwestern Medical Center, Dallas, Texas 75390

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Sugita, Hiroki, Masao Kaneki, Eriko Tokunaga, Michiko Sugita, Chieko Koike, Shingo Yasuhara, Ronald G. Tompkins, and J. A. Jeevendra Martyn. Inducible nitric oxide synthase plays a role in LPS-induced hyperglycemia and insulin resistance. Am J Physiol Endocrinol Metab 282: E386–E394, 2002; 10.1152/ajpendo.00087.2001.—The molecular mechanisms underlying endotoxin-induced insulin resistance remain unclear. Endotoxin or lipo polysaccharide (LPS) injection is a potent stimulator of inducible nitric oxide synthase (iNOS). This study in rats, using the specific iNOS inhibitor aminoguanidine, investigated the role of iNOS in endotoxin-induced hyperglycemia and insulin resistance. LPS injection led to hyperglycemia, insulin resistance, and increased iNOS protein expression and activity. Aminoguanidine prevented LPS-induced hyperglycemia without affecting insulin levels or iNOS expression. Aminoguanidine attenuated the LPS-induced insulin resistance, reflected by the requirement for a higher glucose infusion rate to maintain euglycemia during a hyperinsulinemic clamp study. Aminoguanidine completely blocked the LPS-elevated hepatic glucose output and also inhibited LPS-induced increases in hepatic glycogen phosphorylase activities and phosphoenolpyruvate carboxykinase (PEPCK) mRNA expression, key enzymes for glycolysis and gluconeogenesis, respectively. Thus, these data demonstrate an important role for iNOS in LPS-induced insulin resistance, evidenced by the attenuation of LPS-induced hyperglycemia and reversal of increased hepatic glucose output by aminoguanidine. The protective effect of aminoguanidine on insulin resistance is probably by attenuation of hepatic glucose output via its inhibition of key enzymes for glycolysis and gluconeogenesis, including glycogen phosphorylase and PEPCK.

endotoxin; hepatic glucose output; euglycemic hyperinsulinemic clamp; aminoguanidine; glycogen phosphorylase; phosphoenolpyruvate carboxykinase

PATIENTS with, and animal models of, sepsis or endotoxemia exhibit many metabolic alterations, including attenuated responsiveness to insulin, whose actions include stimulation of peripheral glucose uptake (25, 53, 55), glycogen synthesis (55), protein synthesis (23), inhibition of glycolysis (26, 43), and gluconeogenesis (7). Attenuated responsiveness to the metabolic actions of insulin is termed insulin resistance. These metabolic alterations also exacerbate infection, retard recovery from disease, and lead to muscle wasting. Despite intensive investigation for a number of years, the molecular events responsible for insulin resistance in many pathological states, including stress and inflammation, still remain uncharacterized. Endotoxemia is a pathological state associated with release of cytokines, systemic inflammation, hyperglycemia, insulin resistance, and the hypercatabolic state (43, 53), which is related to the decreased anabolic effects of insulin (8, 48).

Bacterial lipopolysaccharide (LPS) is a key mediator of many of the host responses resulting from gram-negative bacteremia and sepsis; it induces many genes involved in the immune, inflammatory, and acute phase responses. Among those genes, inducible nitric oxide synthase (iNOS) has been implicated in both protective (e.g., bactericidal) and detrimental host responses to sepsis and endotoxemia (46, 57, 59). Mice with knockout of iNOS are resistant to LPS-induced hypotension (13, 27, 51). Consistent with the data on iNOS knockout mice, an iNOS inhibitor, aminoguanidine (AG), reduced mortality after LPS administration to wild-type mice (51). Other iNOS-involved adverse host responses to endotoxin include vascular hyperpermeability (12), myocardial dysfunction (1), liver damage (24), and gut barrier failure (10, 32).

LPS injection was associated with induction of iNOS. These conditions were associated with release of cytokines and systemic inflammation, leading to an increased hepatic glucose output via its inhibition of key enzymes for glycogenolysis and gluconeogenesis, respectively. Thus, these data demonstrate an important role for iNOS in LPS-induced insulin resistance, evidenced by the attenuation of LPS-induced hyperglycemia and reversal of increased hepatic glucose output by aminoguanidine. The protective effect of aminoguanidine on insulin resistance is probably by attenuation of hepatic glucose output via its inhibition of key enzymes for glycolysis and gluconeogenesis, including glycogen phosphorylase and PEPCK.

Address for reprint requests and other correspondence: J. A. J. Martyn (E-mail: jmartyn@etherdome.mgh.harvard.edu) and M. Kaneki (E-mail: mkaneki@helix.mgh.harvard.edu), Dept. of Anesthesia and Critical Care, Massachusetts General Hospital, 55 Fruit St., Boston, MA 02114.
tions include infection (6), sepsis, burn (33), autoimmun
deficiency syndrome (36), and elevated levels of tumor necrosis factor-α (TNF-α) (2, 19) and endotoxin (17, 18). Third, treatment of muscle cells with LPS, TNF-α, and interferon-γ induced iNOS expression and impaired insulin-stimulated glucose uptake; the iNOS inhibitor AG reversed the altered glucose uptake (2). Similarly, TNF-α attenuated insulin-stimulated p70S6K activation, a downstream molecule of insulin signaling, by inducing iNOS expression in pancreatic β-cells (19). Consistent with these findings, nitric oxide (NO) donor impaired insulin-stimulated glucose uptake in muscle cells (17), or decreased glycogen synthesis and increased glycogen phosphorylase activity in primary hepatocytes (44). Furthermore, NO donor administration caused hyperglycemia in dogs (30) and insulin resistance in elderly healthy subjects (31). Finally, the iNOS inhibitor AG inhibited the development of hyperglycemia in genetically obese, diabetic (fa/fa) rats (42) and lowered blood glucose levels in genetically obese, diabetic (db/db) mice (35).

Thus these previous findings reveal a close relationship between iNOS, NO, and insulin resistance. However, the effects of iNOS inhibitor on insulin sensitivity have not been studied in vivo as yet, and the potential involvement of iNOS in disrupted glycemic control in endotoxosis or sepsis remains unexplored. This study, therefore, tested the hypothesis that the endotoxin-induced increased expression of iNOS plays a role in hyperglycemia and insulin resistance. This aim was achieved by use of a specific, competitive inhibitor of iNOS, AG. The effects of AG on glycemic control in vivo and on proglycemic enzymes in the liver were examined.

**EXPERIMENTAL PROCEDURES**

*Animals.* Adult male Sprague-Dawley rats (176–200 g), purchased from Taconic Farms (Germantown, NY), were used for the study. The Institutional Animal Care Committee approved the study protocol. The Association accredits the animal care facility for Assessment and Accreditation of Laboratory Animal Care. The rats were housed in mesh cages in a room maintained at 25°C and illuminated in 12:12-h light-dark cycles; they were provided with standard rodent chow and water ad libitum.

**Measurement of blood glucose and insulin levels in LPS- and AG-administered rats.** After overnight fasting (18 h), each of four groups of rats received the injection of saline alone (group I), saline plus AG (group II), LPS plus saline (group III), or LPS plus AG (group IV). Just after the intraperitoneal injection of LPS [20 mg/kg, derived from *Escherichia coli* Serotype 055:B5 (Sigma, St. Louis, MO)] or saline, AG (50 or 500 mg/kg, Sigma) or saline was injected intraperitoneally. Blood samples were obtained from the tail vein at 0, 60, 90, 120, 150, 180, and 240 min after LPS injection to measure glucose and insulin levels. Blood glucose was measured by an Elite glucometer (Bayer, Elkhart, IN). Insulin concentrations in plasma, obtained from blood with heparin sodium, were determined using an ELISA kit (Crystal Chem, Chicago, IL). The dose of AG (500 mg/kg) was chosen on the basis of the previous studies. In those studies, the same or similar doses of AG were used with efficacy to improve glycemic control in genetically obese, diabetic rats (42) or to inhibit iNOS activities in animal models with various diseases, including endotoxosis (39, 58), pancreatic β-cell damage (42, 47), diabetes (both types I and II), wound healing (41), and radiation-induced lung injury (50).

**Euglycemic hyperinsulinemic clamp study.** The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg), and catheters (PE-50, PE-10; Becton-Dickinson, Sparks, MD) were implanted into the right jugular vein and the left carotid artery, as described previously (15). The catheters were filled with saline containing heparin sodium. At 5–6 days after the catheter placement, euglycemic hyperinsulinemic clamp studies were performed in awake, unrestrained rats in combination with the infusion of [3H]glucose (Amersham Pharmacia, Piscataway, NJ) and 2-deoxy-[14C]glucose (Amersham Pharmacia), as previously described (54). After an overnight fast, the infusion of human regular insulin (Humulin R, Eli Lilly, Indianapolis, IN) was started and maintained at the constant rate (20 mU·min⁻¹·kg⁻¹) via the venous cannula. Blood samples were taken from the arterial catheter for the measurement of blood glucose levels at 5- to 10-min intervals. Euglycemia was maintained by varying the infusion rate of 50% dextrose solution. At the inception of insulin infusion, [3H]glucose (6 µCi) was administered by the intravenous bolus injection, followed by the constant infusion of 0.1 µCi/min. At 120 min after the initiation of insulin infusion, 2-deoxy-[14C]glucose (8 µCi) was administered by intravenous bolus injection. At 120, 130, 140, and 150 min after the inception of insulin infusion, blood samples were collected to measure [3H]glucose and 2-deoxy-[14C]glucose levels. LPS and/or AG was administered at 60 min after the initiation of insulin infusion. At 150 min after the initiation of insulin infusion, the rats were anesthetized with the intravenous injection of pentobarbital sodium (60 mg/kg), and then gastrocnemius muscle (skeletal muscle), liver, and epididymal fat (adipose tissue) were taken and frozen in liquid nitrogen. All of the tissue samples were stored at −80°C until assayed. The rats were euthanized with an overdose of pentobarbital (200 mg/kg ip).

**Measurement of radiolabeled glucose concentrations in plasma and tissues.** To determine the radioactivity of [3H]glucose and 2-deoxy-[14C]glucose in blood, aliquots of plasma (50 µl) obtained with heparin sodium were deproteinized with 100 µl of Ba(OH)₂ (0.3 N) and ZnSO₄ (0.3 N) and then centrifuged. The radioactivity of 3H and 14C in the protein-free supernatants of Ba(OH)₂ and ZnSO₄ precipitates was measured with a liquid scintillation counter. To eliminate tritiated water, the supernatants were evaporated to dryness for the measurement of [3H]glucose. Glucose specific activities of plasma were calculated by dividing 3H and 14C radioactivity in the dried, reconstituted aliquots by amount of glucose. To measure the radioactivity of 2-deoxy-[14C]glucose in tissues, the frozen tissue samples were digested with 1 ml of 1 M NaOH at 60°C for 1 h and then neutralized with 1 ml of 1 M HCl. Aliquots of 150 µl of digested tissue samples were mixed with 750 µl HClO₄ (4% wt/vol) and then centrifuged. Thereafter, the radioactivity of 14C in the supernatants was measured. Tissue glucose uptake (Rg) was calculated as follows (56)

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R_g (\text{mg·kg}^{-1}·\text{min}^{-1}) = C_p \times C_m \times \int_0^{60} C_v (t) \, dt
\]

where \(C_p\) is the steady-state plasma glucose concentration, \(C_m\) is the tissue accumulation of 2-deoxy-[14C]glucose per
unit mass, and $C_p(t)$ is the plasma 2-deoxy[14C]glucose concentration.

**Calculation of hepatic glucose output.** Hepatic glucose output was determined as previously described (38). Briefly, glucose appearance rate was calculated as the ratio of the infusion rate of $[^3H]$glucose specific activity (disintegration/min) and the steady-state plasma $[^3H]$glucose concentration. The rate of hepatic glucose output was calculated as glucose appearance rate minus the glucose infusion rate (hepatic glucose output = glucose appearance rate – glucose infusion rate).

**Measurement of glyogen phosphorylase activity.** The activities of glycogen phosphorylase in liver were measured as described previously (45). After 10 min at 37°C, the reaction was terminated with 500 μl of stop solution containing 20 mM HEPEs (pH 5.5) and 2 mM EDTA; it was applied to a 1-ml column of Dowex AG50Wx8 (Na+ form) (Bio Rad Laboratories), which was eluted with 500 μl of water. l-[14C]citrulline was quantified by a liquid scintillation counter. iNOS activity was measured as Ca2+-independent NOS activities in the presence of 1 mM EGTA, being determined from the difference between samples with and without NOS inhibitor L-nonsymethyl-l-arginine (l-NAME, 10 mM; Calbiochem, San Diego, CA).

**RNase protection assay.** Total RNA was isolated from liver using the QuickPrep Total RNA Extraction Kit (Amersham Pharmacia) according to the manufacturer’s instructions. The fragment of cDNA for phosphoenolpyruvate carboxykinase (PEPCK) was obtained from rat total liver RNA (Ambion, Austin, TX) by RT-PCR by use of the primers of 5'-ggagcaatctgagatccagtttct-3' and 5'-gaacagcactcagttataactc-3', which was then subcloned into the pCR2.1 vector (Invitrogen, Carlsbad, CA). An antisense riboprobe was transcribed with T7 RNA polymerase (Ambion) by using [32P]UTP (Amersham Pharmacia). RNase protection assays were carried out by using the RPA III kit (Ambion). Briefly, the riboprobe for PEPCK was hybridized with 20 μg of total RNA extracted from rat liver at 42°C for 18 h followed by RNase A/T1 digestion at 37°C for 30 min. Protected fragments were heat denatured and separated on 5% denaturing polyacrylamide gels, dried, and exposed to film (Kodak BioMax MR, Rochester, NY). A rat β-actin antisense probe was used as a control. A cDNA fragment for β-actin was purchased from Ambion. Radioactive signals were scanned and quantitated by use of National Institutes of Health Image 1.61.

**Statistical analysis.** The data were compared using one-way ANOVA followed by Fisher’s protected least significant difference test. The null hypothesis was rejected when $P < 0.05$. All values are expressed as means ± SE.

**RESULTS**

Effects of AG in LPS-induced hyperglycemia and iNOS induction. LPS administration led to hyperglycemia within 60 min. The maximal hyperglycemic response was observed at 90 min after LPS injection. The 50 mg/kg dose of AG did not change blood glucose levels (data not shown). However, 500 mg/kg of AG treatment abrogated the LPS-induced hyperglycemia but did not affect blood glucose levels in LPS-naive (saline-injected) rats (Fig. 1A). LPS increased the insulin level. AG failed to modulate insulin levels in both saline- and LPS-injected rats (Fig. 1B), suggesting that AG improved glycemic control in the LPS-injected rats through an effect on peripheral insulin sensitivity, and not by increasing insulin secretion from pancreatic β-cells.

iNOS protein expression in the liver was undetectable in saline-injected rats but was markedly induced by...
in LPS-injected rats. AG did not affect iNOS expression (Fig. 2A). Consistent with the induction of iNOS protein expression, LPS administration caused a significant increase in iNOS activity. AG completely inhibited the LPS-induced increase in iNOS activity but did not affect iNOS activity in saline-injected (saline-injected) rats (Fig. 2B).

Effects of LPS and AG on whole body insulin sensitivity. A euglycemic hyperinsulinemic clamp study was performed to examine the effects of AG (500 mg/kg) on LPS-induced insulin resistance. Steady-state glucose level was achieved during the 60- to 90-min period after LPS injection. During steady-state glucose infusion, blood glucose levels did not differ among the four groups. The average blood glucose levels during the steady state of the animals treated with saline alone (LPS naive), AG alone, LPS alone, and LPS + AG were 115.1 ± 5.4 (SE), 109.8 ± 3.8, 117.7 ± 4.3, and 122.1 ± 4.9 mg/dl, respectively. Whole body insulin sensitivity was judged by the glucose infusion rate required to maintain steady-state blood glucose levels between 60 and 90 min after LPS injection. The glucose infusion rate to maintain steady-state glucose level was significantly decreased in the rats administered LPS. AG reversed significantly the LPS-induced decrease in the requirement of glucose infusion rate. AG, when administered to LPS-naive (saline-injected) rats, also decreased glucose infusion rate significantly (Fig. 3).

Effects of AG on insulin-stimulated glucose uptake in tissues. LPS significantly reduced tissue glucose uptake by skeletal muscle during the euglycemic hyperinsulinemic clamp. AG did not affect skeletal muscle glucose uptake in either saline- or LPS-injected rats (Fig. 4). Glucose uptake by liver was unaltered by LPS;
tissues studied. Values are means ± SE; n = 4–7/group. *P < 0.05 vs. rats treated with saline alone; §P < 0.05 vs. rats treated with saline.

the trend for decreased uptake by liver was not statistically significant. AG failed to alter insulin-stimulated glucose uptake by the liver. In contrast to liver and muscle, glucose uptake in adipose tissue was relatively low in all groups, with no differences observed between groups with and without AG.

Effects of AG on hepatic glucose output. Hepatic glucose output was calculated as glucose appearance rate minus the infusion rate. Hepatic glucose output was almost zero in saline-treated (LPS-naive) rats, because a large infusion dose of insulin suppressed hepatic glucose output completely. AG did not alter hepatic glucose output in control animals (LPS naive). LPS significantly increased hepatic glucose output. AG effectively decreased LPS-induced hepatic glucose output to the level of controls (Fig. 5). This, therefore, contrasts with the ineffectiveness of AG on glucose uptake by liver and muscle in LPS-injected rats.

Effects of AG on glycogen phosphorylase activity and PEPCK mRNA expression. The data we have described suggest that the protective effects of AG on LPS-induced hyperglycemia and whole body insulin resistance might be accounted for mainly by inhibition of LPS-induced elevation of hepatic glucose output. Augmented hepatic glucose output is attributable to increased glycogenolysis and/or gluconeogenesis. Therefore, we examined the effects of AG on glycogen phosphorylase and PEPCK, key enzymes for glycogenolysis and gluconeogenesis, respectively. LPS upregulated both total glycogen phosphorylase activity and glycogen phosphorylase-a activity. AG restored both phosphorylase activities to the basal levels (Fig. 6, A and B). In contrast, phosphorylase activities were unaltered by AG in saline-injected rats (controls). The RNase protection assay demonstrated that LPS significantly increased PEPCK mRNA expression levels. AG inhibited LPS-induced increase in PEPCK expression (Fig. 7, A and B) but did not affect PEPCK mRNA level in saline-injected rats. The expression levels of house-keeping gene β-actin mRNA were unaltered by either LPS or AG.

**DISCUSSION**

The detrimental effects of LPS on glucose metabolism observed in the present study confirm the previous reports of altered glucose homeostasis in septic patients and animals administered LPS. The adverse effects on glucose homeostasis induced by LPS include hyperglycemia (Fig. 1) (14, 21, 40), whole body insulin resistance, as judged by the glucose infusion rate dur-

![Fig. 3. Effects of LPS and AG on glucose infusion rate during euglycemic hyperinsulinemic clamp. The steady-state blood glucose level was achieved between 60 and 90 min after LPS injection. During the steady state, blood glucose levels did not differ among the 4 groups of rats (saline alone, saline + AG, LPS + saline, and LPS + AG). Glucose infusion rate to maintain normal blood glucose levels reflects whole body insulin sensitivity. LPS injection caused a profound reduction in glucose infusion rate; that is, insulin sensitivity decreased. AG significantly increased the glucose infusion rate in LPS-injected rats, indicating improved insulin sensitivity. On the other hand, AG decreased glucose infusion rate in saline-injected rats, which suggests that the protective effects of AG on LPS-induced hyperglycemia and whole body insulin resistance might be accounted for mainly by inhibition of LPS-induced elevation of hepatic glucose output. Augmented hepatic glucose output is attributable to increased glycogenolysis and/or gluconeogenesis. Therefore, we examined the effects of AG on glycogen phosphorylase and PEPCK, key enzymes for glycogenolysis and gluconeogenesis, respectively. LPS upregulated both total glycogen phosphorylase activity and glycogen phosphorylase-a activity. AG restored both phosphorylase activities to the basal levels (Fig. 6, A and B). In contrast, phosphorylase activities were unaltered by AG in saline-injected rats (controls). The RNase protection assay demonstrated that LPS significantly increased PEPCK mRNA expression levels. AG inhibited LPS-induced increase in PEPCK expression (Fig. 7, A and B) but did not affect PEPCK mRNA level in saline-injected rats. The expression levels of house-keeping gene β-actin mRNA were unaltered by either LPS or AG.

**DISCUSSION**

The detrimental effects of LPS on glucose metabolism observed in the present study confirm the previous reports of altered glucose homeostasis in septic patients and animals administered LPS. The adverse effects on glucose homeostasis induced by LPS include hyperglycemia (Fig. 1) (14, 21, 40), whole body insulin resistance, as judged by the glucose infusion rate dur-
ing euglycemic hyperinsulinemic clamp (Fig. 3) (25, 55), attenuated insulin-stimulated glucose uptake in skeletal muscle (Fig. 4) (25, 53, 55), elevated hepatic glucose output (Fig. 5) (40), increased glycogen phosphorylase activities (Fig. 6) (26, 43), and increased PEPCK expression (Fig. 7) (7).

The salient new findings of the present study are that 1) the iNOS inhibitor AG decreased LPS-induced hyperglycemia and inhibited partially but significantly LPS-induced whole body insulin resistance (Figs. 1, 3); 2) AG completely blocked LPS-induced elevation in hepatic glucose output but did not alter insulin-stimulated glucose uptake in tissues (Figs. 4 and 5); 3) the LPS-mediated increases in glycogen phosphorylase activities (Fig. 6) and PEPCK mRNA expression (Fig. 7) were reversed by AG. The beneficial effects of AG were observed in LPS-injected rats in which iNOS was induced, but not in saline-injected rats where iNOS was undetectable (Fig. 1–7). On the basis of these data, one can conclude that iNOS plays a pivotal role in hyperglycemia and hepatic insulin resistance of endotoxin and that AG inhibits LPS-induced hyperglycemia and hepatic insulin resistance mainly by reversing the detrimental effects of LPS on hepatic glucose output. The abrogation of LPS-induced increases in glycogen phosphorylase activities and PEPCK expression probably contributed to the protective effects of AG. These findings are consistent with other reports that iNOS plays a key role in the deleterious host responses to endotoxin.

The protective effects of the specific iNOS inhibitor (Fig. 5) contrasts with an earlier study demonstrating that nonselective iNOS inhibition of all NO synthase

Fig. 6. Effects of LPS and AG on glycogen phosphorylase activity. LPS upregulated the activities of both total glycogen phosphorylase and glycogen phosphorylase-a in liver. AG inhibited the LPS-induced activation of both total glycogen phosphorylase and glycogen phosphorylase-a (A and B). On the other hand, AG did not alter glycogen phosphorylase activities in saline-injected rats. Neither LPS nor AG affected the ratio of the activities of glycogen phosphorylase-a and total glycogen phosphorylase (C). Values are means ± SE; n = 4–7. *P < 0.01 vs. rats treated with saline alone; †††P < 0.01 vs. rats treated with LPS + saline. CPM, counts/min.

Fig. 7. Effects of LPS and AG on phosphoenolpyruvate carboxykinase (PEPCK) mRNA expression. PEPCK RNA expression in liver was examined by RNase protection assay. LPS significantly increased PEPCK mRNA expression, and AG inhibited LPS-induced increase in PEPCK mRNA expression. On the other hand, β-actin mRNA expression did not differ in liver among the rats treated with or without LPS or AG. Top: typical blots of RNase protection assay. Values are means ± SE; n = 4–7. *P < 0.01 vs. rats treated with saline alone; †††P < 0.01 vs. rats treated with LPS + saline.
isoenzymes (iNOS, endothelial NOS, and neuronal NOS) did not cause a statistically significant decrease in elevated hepatic glucose output in LPS-administered pigs (49). The nonspecific nature of the NOS inhibition may account for the lack of significant difference or effectiveness.

Although AG is known to be capable of inhibiting advanced glycation end product (AGE) formation as well as iNOS, the doses of AG needed to inhibit AGE formation and iNOS differ substantially. Relatively low doses (50 mg/kg body wt) of AG are sufficient to inhibit AGE formation when administered to rodents. In contrast, higher doses of AG (125–500 mg/kg body wt) are needed to inhibit LPS-induced iNOS activity (39). There is no report of increased AGE formation induced by LPS. Taken together, on the basis of our data that the low dose of AG (50 mg/kg body wt) was ineffective but that the higher dose of AG (500 mg/kg body wt) was required to inhibit LPS-induced hyperglycemia, AG is likely to exert its protective effects by inhibiting iNOS activity but not by blocking AGE formation. In addition, our preliminary experiment (data not presented) showed that another iNOS inhibitor, S-methylthiourea (SMT), also significantly decreased blood glucose levels in LPS (3 mg/kg iv)-injected rats, despite the fact that SMT does not inhibit AGE formation. Blood glucose levels in rats treated with LPS + saline, LPS + SMT (5 mg/kg), and LPS + SMT (50 mg/kg) were 106.3 ± 14.3 (SE), 95.7 ± 9.7, and 56.3 ± 0.8 mg/dl, respectively; P < 0.01, LPS + saline vs. LPS + SMT (50 mg/kg). These data are supportive of a role for iNOS in LPS-induced derangements in glucose metabolism.

LPS impaired insulin-stimulated glucose uptake in skeletal muscle, the major tissue for insulin-mediated glucose uptake (Fig. 4). LPS-induced alterations in the insulin-stimulated glucose uptake were not observed, however, in liver and adipose tissue. This selective impairment in skeletal muscle is in accordance with the previous reports in septic rats (20, 22) but not with another study showing that LPS impaired insulin-stimulated glucose uptake not only in skeletal muscle but also in liver and adipose tissue (25). This apparent discrepancy may be explained by the differences in the experimental design, such as insulin infusion rates, the body weight of animals, and the doses of endotoxin. AG was ineffective in reversing the attenuated insulin-stimulated glucose uptake by skeletal muscle in LPS-injected rats (Fig. 4). Therefore, LPS seems to exert its adverse effects on insulin-stimulated glucose uptake in skeletal muscle by an iNOS-independent mechanism.

LPS-induced insulin resistance in skeletal muscle is postulated to be mediated by a β-adrenergic mechanism, a conclusion that was based on the protective effects of the β-adrenergic antagonist propranolol (20). AG administration resulted in a modest but significant decrease in glucose infusion rate during the hyperinsulinemic euglycemic clamp in control (LPS-naïve) rats, whereas it induced a significant increase in glucose infusion rate in LPS-injected rats (Fig. 3). The biological significance and mechanism for the decreased glucose infusion rate in controls during AG therapy are unclear. However, the AG-induced decrease in glucose infusion rate in controls is unlikely to be attributable to the effects of iNOS, because iNOS was not induced (Fig. 2); neither glucose uptake in skeletal muscle, liver, and adipose tissue nor hepatic glucose output was affected by AG in control rats (Figs. 4 and 5).

Increased hepatic glucose output is responsible for fasting hyperglycemia (48). Because AG did not alter insulin levels, one can conclude that the normoglycemic action of AG is not via pancreatic β-cells to increase insulin secretion but a peripheral action on target tissues. The attenuation of elevated hepatic glucose output by the iNOS inhibitor accounts for its potential normoglycemic effects after a hyperglycemic response to LPS. In contrast, the relatively modest amelioration of glucose infusion rate by AG in LPS-treated animals during euglycemic hyperinsulinemic clamp may be explained by the failure to mitigate the impaired glucose uptake in skeletal muscle. When these observations are taken together, therefore, one can conclude that the mechanisms underlying LPS-induced insulin resistance differ between tissues, and that iNOS-dependent and iNOS-independent mechanisms may play major roles in the derangement in insulin actions in liver and skeletal muscle of LPS-injected rats, respectively.

Glycogenolysis and gluconeogenesis are the two major biochemical pathways for hepatic glucose production. Glycogen phosphorylase is a key enzyme responsible for glycogenolysis. Glycogenolysis is increased by LPS (5, 28). A recent study (29) demonstrating that the inhibition of glycogen phosphorylase lowered hyperglycemia of genetically obese, diabetic (ob/ob) mice emphasizes the important role of glycogen phosphorylase in hyperglycemia in diabetes mellitus. In our study, the parallel relationship between induction of glycogen phosphorylase activity and hyperglycemia and the normalization of the enzyme activity with AG and attenuation of hyperglycemia suggest a role for glycogen phosphorylase activity in the hyperglycemic response to LPS. However, little is known about how glycogenolysis and glycogen phosphorylase activity are upregulated by endotoxin.

Glycogen phosphorylase, when phosphorylated by cAMP-dependent glycogen phosphorylase kinase, becomes activated and is called “glycogen phosphorylase-a”; the dephosphorylated form is inactive and referred to as “glycogen phosphorylase-b.” Glycogen phosphorylase-b (inactive form) is subject to allosteric activation with AMP, and in the presence of AMP, glycogen phosphorylase-b is as active as glycogen phosphorylase-a. Thus the activities measured in the presence and absence of AMP represent those of total (a + b) glycogen phosphorylase and glycogen phosphorylase-a alone, respectively. The present study reveals that LPS increased the activities of both total and glycogen phosphorylase-a without affecting the ratio between the total enzyme and the phosphorylated fraction of the enzyme (Fig. 6). These results indicate that the activa-
tion of glycogen phosphorylase by LPS is not mediated by glycogen phosphorylase kinase or the phosphorylation status of the enzyme.

It is noteworthy that β-adrenergic agonists activate and insulin inactivates glycogen phosphorylase by altering phosphorylation status, namely the ratio of phosphorylase-a to total phosphorylase (a/total(a) [34, 52]). In this study, LPS and AG modulated the enzyme activities, with no change in a/total(a) (Fig. 6C). This finding is consistent with the assumption that LPS-induced hepatic insulin resistance is β-adrenergic independent. It is also suggested that phosphorylase activation might be one of the primary events of hepatic insulin resistance, rather than just a consequence secondary to insulin resistance, considering that phosphorylase activation resulting from insulin resistance, namely, decreased insulin action, would be accompanied by an increase in a/total(a). Combined with a previous report showing that an NO donor increased glycogen phosphorylase activity and glycogenolysis (34, 44, 52), the protective effects of AG on LPS-induced glycogen phosphorylase activation in the present study indicate that LPS upregulated glycogen phosphorylase activity by inducing iNOS.

Gluconeogenesis is upregulated in sepsis in the fasting condition (9). PEPCK, a rate-limiting enzyme for gluconeogenesis, is known to be induced by LPS and sepsis (7). The activities of PEPCK are considered to be regulated by its expression levels, and increased expression of PEPCK has been assumed to play a critical role in elevated hepatic glucose production in sepsis and endotoxiosis, as well as type 2 diabetes. Our results of the inhibitory effects of AG on PEPCK expression (Fig. 7) suggest that iNOS may also be involved in PEPCK induction by LPS.

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