Histidine Augments the Suppression of Hepatic Glucose Production by Central Insulin Action

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Glucose intolerance in type 2 diabetes is related to enhanced hepatic glucose production (HGP) due to the increased expression of hepatic gluconeogenic enzymes. Previously, we revealed that hepatic STAT3 decreases the expression of hepatic gluconeogenic enzymes and suppresses HGP. Here, we show that increased plasma histidine results in hepatic STAT3 activation. Intravenous and intracerebroventricular (ICV) administration of histidine-activated hepatic STAT3 reduced G6Pase protein and mRNA levels and augmented HGP suppression by insulin. This suppression of hepatic gluconeogenesis by histidine was abolished by hepatic STAT3 deficiency or hepatic Kupffer cell depletion. Inhibition of HGP by histidine was also blocked by ICV administration of a histamine H_1 receptor antagonist. Therefore, histidine activates hepatic STAT3 and suppresses HGP via central histamine action. Hepatic STAT3 phosphorylation after histidine ICV administration was attenuated in histamine H_1 receptor knockout (Hrh1KO) mice but not in neuron-specific insulin receptor knockout (NIRKO) mice. Conversely, hepatic STAT3 phosphorylation after insulin ICV administration was attenuated in NIRKO but not in Hrh1KO mice. These findings suggest that central histidine action is independent of central insulin action, while both have additive effects on HGP suppression. Our results indicate that central histidine/histamine-mediated suppression of HGP is a potential target for the treatment of type 2 diabetes. ***Diabetes*** **62:2266–2277, 2013**

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Increased glucose production in type 2 diabetes is caused by elevated gluconeogenesis in the liver (1), while in actual clinical settings, treatment for diabetes includes remedies, such as metformin, that suppress hepatic gluconeogenesis (2). Hepatic gluconeogenesis is controlled by regulating the gene expression of gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) (2). In fact, gene expression of hepatic gluconeogenic enzymes is upregulated in obese animal models of diabetes, including *db/db* mice lacking leptin receptors (3,4), while suppression of gluconeogenic gene expression improves glucose tolerance in *db/db* mice (4–6).

Hormonal and nutrient changes regulate the expression of hepatic gluconeogenic genes by a central-mediated mechanism in addition to direct hepatic action (7,8). Insulin, the most important regulatory factor in gluconeogenesis, suppresses hepatic gluconeogenic gene expression via a central indirect mechanism through brain insulin receptors and via direct action on hepatic insulin receptors (9,10). In rodent studies, the intracerebroventricular (ICV) administration of insulin downregulates gluconeogenic gene expression in the liver and suppresses hepatic glucose production (HGP) (10,11). Conversely, HGP is increased in hypothalamic insulin receptor knockdown rats and neuron-specific insulin receptor knockout (NIRKO) mice (9,10). In addition, inhibition of phosphatidylinositol 3-kinase (PI3-K) in the insulin-signaling pathway in the hypothalamus inhibits the central insulin-dependent suppression of HGP (10). Previously, we revealed the importance of signal transducer and activator of transcription-3 (STAT3) in the central insulin-mediated suppression of gluconeogenesis in the liver. In fact, STAT3 is activated through phosphorylation, which is induced by glucose load in an insulin-dependent manner and also by the ICV administration of insulin (9). Further, liver-specific STAT3-deficient (LST3KO) mice exhibit a defect in the central insulin-mediated suppression of HGP (9), suggesting that hepatic STAT3 plays an important role in this process (9). We also revealed that the upregulation of liver-specific interleukin-6 (IL-6) expression is required for the activation of hepatic STAT3 by central insulin (9).

Changes in nutrients are known to affect hepatic gluconeogenesis via central action similarly to insulin (7,8). Glucose and long-chain fatty acids are known to suppress glucose production in the liver after ICV injection (12,13). Recent studies have reported that plasma amino acid levels are also closely associated with glucose metabolism. In fact, in individuals without diabetes and those with new-onset type 2 diabetes, fasting and 2-h blood glucose levels

after glucose loading correlate positively with the blood concentration of branched-chain amino acids alanine, phenylalanine, and tyrosine and negatively with the concentration of histidine and glutamine (14). However, the function of these amino acids in the central-mediated regulation of gluconeogenesis remains to be fully elucidated, while the branched-chain amino acid leucine reportedly suppresses HGP when administered to the hypothalamus (15). In the current study, we found that plasma histidine acts as a nutrient that suppresses hepatic gluconeogenesis via central-mediated hepatic STAT3 activation.

RESEARCH DESIGN AND METHODS

Experiments using mice were conducted in accordance with the guidelines for the care and use of laboratory animals of Kanazawa University. Male mice (8–10 weeks old) were housed under a 12-h light-dark cycle with free access to food and water. Wild-type C57BL/6J Slc mice were purchased from Japan SLC (Shizuoka, Japan), and histamine H_1 receptor knockout (Hrh1KO) (16) mice were from Oriental Bio Service (Kyoto, Japan). We generated LST3KO mice by crossing albumin-cre and floxed-STAT3 mice (5), while NIRKO mice were generated by crossing Nestin-cre and floxed–insulin receptor mice (17). Floxed-STAT3 and floxed–insulin receptor mice were used as the corresponding controls.

Administration of protein extracts and amino acids. Chicken meat extract was prepared as a protein source for gavage administration. Shredded chicken meat was cooked in a pressure cooker for 270 min and strained, the lipids were removed by centrifugation, and the protein concentration was adjusted. Snakehead fish meat was processed in the same way and used as a low-histidine (Low-His) protein source that did not increase blood histidine levels. After 16 h of fasting, the mice were orally administered protein at 2 g/kg body wt via a feeding tube. Histidine or each amino acid was administered at 0.5 mmol/kg body wt i.p. or intravenously at the concentrations shown in Figs. 2*A* and 5*G* after a 16-h fast.

Tolerance tests and biochemical and endocrine testing. A glucose, pyruvate, or fructose tolerance test was performed after a 16-h fast. For the glucose tolerance test, histidine was injected intraperitoneally at 5 min before injection of 2 g/kg body wt glucose (Otsuka Pharmaceutical, Tokushima, Japan). For the pyruvate or fructose tolerance test, histidine was injected twice at 120 and 5 min before 1 g/kg body wt sodium pyruvate or fructose, respectively (Sigma-Aldrich, St. Louis, MO). Blood glucose levels were measured using a GLUCOCARD G+ Meter (Arkray, Kyoto, Japan). Blood insulin and IL-6 levels were measured using a Mouse Insulin ELISA kit (Shibayagi, Gunma, Japan) or a Quantikine Mouse IL-6 ELISA kit (R&D Systems, Minneapolis, MN). Plasma levels of amino acids were quantified using high-performance liquid chromatography. Hypothalamic histamine levels were measured using a Histamine EIA kit (Bertin Pharma, York, U.K.). Hepatic glycogen levels were measured by the anthrone–sulfuric acid method.

Euglycemic clamp technique. Hyperinsulinemic-euglycemic clamping was performed by injecting the awake and unrestrained mice with human insulin (Eli Lilly, Indianapolis, IN) after a 16-h fast. Blood glucose levels were measured every 10 min and maintained between 90 and 120 mg/dL after intravenous insulin injections were started. Further, between 90 and 120 min after starting insulin administration, we stabilized blood glucose volatility with 20 mg/dL insulin (Supplementary Table 1). We measured the glucose infusion rate (GIR) and plasma [^{3}H]glucose specific activity every 10 min. The R_d under steady-state conditions for plasma glucose concentration was determined from the rate of [^{3}H]glucose infusion divided by the plasma [^{3}H]glucose specific activity. The rate of HGP was obtained from the difference between R_d and GIR. An internal cannula (Plastics One, Roanoke, VA) was inserted into the lateral ventricle, followed by intravenous cannulation 7–10 days later. After 4–6 days of recovery and habituation, a hyperinsulinemic-euglycemic clamp or pancreatic clamp was performed with ICV administration. After a 16-h fast, the mice received an ICV administration of human insulin, histidine, or pyrilamine with an intravenous injection of human insulin and somatostatin, as shown in Fig. 7*A*. Blood glucose levels were maintained between 90 and 120 mg/dL. The mice received 10 nmol/mouse LY294002 (Calbiochem, San Diego, CA), 50 μg/mouse pyrilamine (Wako, Osaka, Japan), 50 nmol/mouse thioperamide (Sigma), pyridylethylamine (TOCRIS Bioscience, Ellisville, MO), or artificial cerebrospinal fluid via the lateral ventricle. For the hyperinsulinemic-euglycemic clamp, pyrilamine was administered to the lateral ventricle (Fig. 5G).

IL-6 neutralizing antibody and Kupffer cell depletion. An IL-6 neutralizing antibody and control IgG (R&D Systems) were injected as previously described (9). Clodronate (LKT Laboratories, St. Paul, MN) was encapsulated into empty liposomes (NOF, Tokyo, Japan), and clodronate-containing liposomes (CLDs) were injected into the mice via the tail vein for two consecutive days to deplete Kupffer cells in the liver. The control mice received empty liposomes. Immunostaining with an anti-Mac-2 antibody (Cedarlane Laboratories, Burlington, Ontario, Canada) was performed to visualize the Kupffer cells.

Western blotting and quantitative PCR. Mouse hepatocytes were isolated as previously described (6). Immunoblotting was performed using anti–phosphorylated (phospho)-STAT3 (Tyr705), anti–phospho-Akt (Thr308), anti–phospho-p70 S6 kinase (Thr389), anti–p70 S6 kinase, anti–phospho-S6 ribosomal protein (Ser235/236), and anti-S6 ribosomal protein antibodies from Cell Signaling Technology (Danvers, MA); anti-STAT3, anti-Akt, anti–G6Pase-α, anti-PEPCK, and anti–insulin receptor β antibodies from Santa Cruz Biotechnology (Santa Cruz, CA); and an anti–β-actin antibody from Sigma. Immunoblot images were representative of at least three independent immunoblot analyses and quantified by densitometry on an LAS-3000 Imager (Fujifilm, Tokyo, Japan).

The results of quantitative PCR were analyzed using the *36B4* gene as an internal control. The primer sequences used in this study are available upon request.

Statistical analysis. Statistical analysis was performed using Student t test and one- or two-way ANOVA followed by post hoc tests, and differences were considered significant for P values <0.05.

RESULTS

Improvement of glucose tolerance after histidine administration. Phosphorylation of hepatic Akt and STAT3 was increased at 120 min after the oral administration of the protein extract (Fig. 1*A*). As in our previous study (9), glucose loading activated hepatic Akt and STAT3, suggesting that both the hepatic and central actions of insulin were induced by glucose loading. In addition, protein administration also augmented the activation of these molecules by glucose loading (Fig. 1*A*). The intake of proteins is known to facilitate the secretion of insulin (18); in fact, the continuous administration of somatostatin, which suppresses the secretion of endogenous insulin, kept Akt phosphorylation at a virtually undetectable level after ingestion of the protein extract (Fig. 1*B*), whereas protein intake increased STAT3 phosphorylation even in the presence of somatostatin (Fig. 1*B*). These findings suggest that hepatic STAT3 activation associated with protein intake is independent of the secretion of endogenous insulin. Because blood amino acid levels are increased after the oral administration of proteins (19), we administered individual amino acids intraperitoneally to investigate the effects of blood amino acid levels on hepatic STAT3 activation. We found that the hepatic STAT3 phosphorylation was increased by the administration of histidine and histidine-related amino acids and dipeptides, such as 1-methyl-histidine, β-alanyl-histidine (carnosine), and β-alanyl-1-methyl-histidine (anserine) (Fig. 1*C*). Therefore, we investigated the importance of histidine and histidine-containing dipeptides in the activation of hepatic STAT3 using a protein and Low-His protein extract. Coincident with the plasma concentration of histidine and other histidine-containing dipeptides (Supplementary Table 2), a milder increase in STAT3 phosphorylation was observed in the Low-His group compared with the protein group (Fig. 1*D*).

Because histidine administration activates hepatic STAT3, we then conducted a glucose tolerance test to investigate the role of histidine in glucose homeostasis. With the intraperitoneal administration of 500 μmol/kg body wt histidine, which maintained the blood concentration of histidine close to that obtained after the oral administration of 2 g/kg body wt protein (Supplementary Table 3), blood glucose levels were significantly decreased at 120 min after glucose loading (Fig. 1*E*). Conversely, there was no change in plasma insulin levels after histidine administration (Fig. 1*F*). Phosphorylation of hepatic STAT3 increased at 120 min after histidine administration, which was further increased

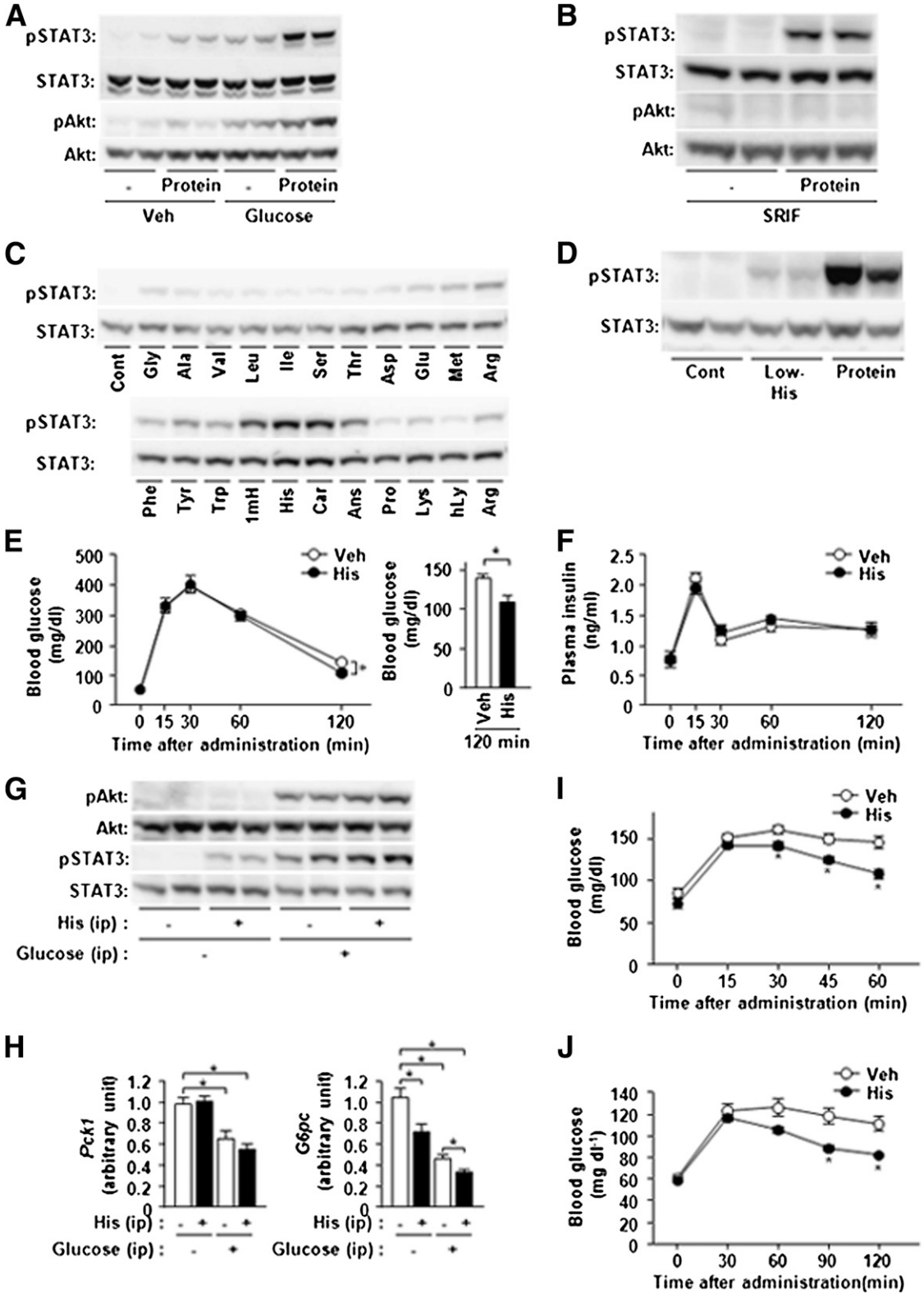

FIG. 1. Phosphorylation of hepatic STAT3 after protein or histidine administration. *A*: Western blotting was performed to analyze the phosphorylation of STAT3 and Akt in the liver at 120 min after the oral administration of protein extract (2 g/kg body wt) and injection of glucose (2 g/kg body wt i.p.) or saline. *B*: Somatostatin was injected continuously at 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ via the jugular vein, and phosphorylation of hepatic STAT3 and Akt was analyzed at 120 min after the oral administration of protein. *C*: Phosphorylation of hepatic STAT3 was analyzed by Western blotting at 120 min after the intraperitoneal administration of amino acids (0.5 mmol/kg). The experiment was performed in quadruplicate ($n = 4$), and samples were combined for Western blot analysis. 1 mH, 1-methyl-histidine; Ans, anserine; Car, carnosine; and hLy, hydroxylysine. *D*: Phosphorylation of STAT3 was analyzed at 120 min after the oral administration of protein or Low-His. *E* and *F*: A glucose tolerance test was performed with the intraperitoneal administration of histidine (His) or saline (Veh). Changes in blood glucose levels (*E*) (*left panel*), blood glucose levels at 120 min after glucose loading (*E*) (*right panel*), and changes in plasma insulin levels (*F*) are shown. *$P < 0.05$ ($n = 10$). *G* and *H*: Western blotting analysis of hepatic STAT3 and Akt phosphorylation (*G*) and quantitative PCR analysis of *Pck1* and *G6pc* gene expression (*H*) were performed at 120 min after the intraperitoneal injection of histidine or saline (-) and glucose (2 g/kg body wt). *$P < 0.05$ ($n = 5$). *I* and *J*: A fructose (*I*) and pyruvate (*J*) tolerance test was performed after the intraperitoneal administration of histidine or saline. *$P < 0.05$ ($n = 10$). Cont, control.

by glucose loading (Fig. 1*G*). While the intraperitoneal administration of histidine alone did not significantly change blood glucose levels, it significantly downregulated the expression of the *G6pc* gene, which encodes G6Pase, with and without glucose loading (Fig. 1*H*). However, the expression of the *Pck1* gene, which encodes PEPCK, did not change significantly after the intraperitoneal administration of histidine alone or with glucose. The administration of fructose and pyruvate is known to increase blood glucose levels mildly through gluconeogenesis (20,21); however, histidine

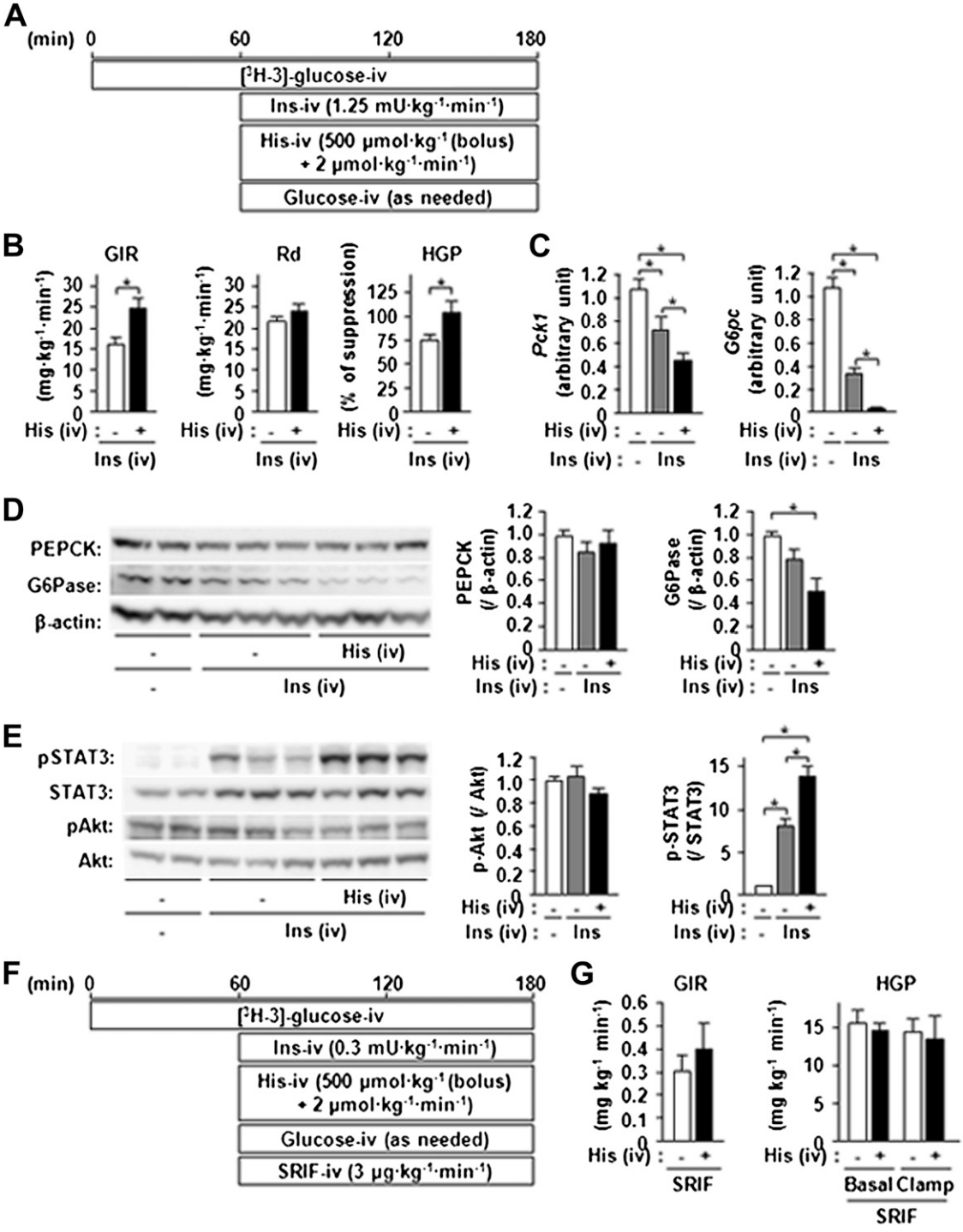

FIG. 2. Augmentation of the insulin-dependent suppression of HGP by histidine. *A*: Schematic of hyperinsulinemic-euglycemic clamping with continuous intravenous administration of histidine (His) or saline (-). *B* and *C*: GIR (*B*, *left panel*), R_d (*B*, *center panel*), and the suppression (%) of HGP (*B*, *right panel*) in the study described in *A*. Quantitative PCR analysis of *Pck1* and *G6pc* gene expression levels in the liver is shown in *C*. *$P < 0.05$ ($n = 7$–9). *D* and *E*: Western blotting was performed to analyze the levels of hepatic PEPCK, G6Pase, and β-actin (*D*) and phosphorylation of STAT3 and Akt (*E*) at 120 min after insulin (Ins) administration in the hyperinsulinemic-euglycemic clamp study (*A*). Quantitation of PEPCK and G6Pase levels is normalized to β-actin (*D*). Quantitation of phospho-Akt and phospho-STAT3 is normalized to Akt and STAT3, respectively (*E*). Data are represented as means ± SE ($n = 7$–9) values. *F*: Schematic of euglycemic-pancreatic clamp tests with continuous intravenous administration of histidine or saline in addition to somatostatin injection. *G*: The GIR (*left panel*) and the suppression (%) of HGP (*right panel*) in the study described in *F* ($n = 5$).

injection attenuated the increase in blood glucose levels after the administration of fructose (Fig. 1*I*) and pyruvate (Fig. 1*J*).

Histidine administration augments the insulin-induced suppression of HGP. We used a hyperinsulinemic-euglycemic clamp technique to investigate the effect of histidine on HGP (Fig. 2*A*). The GIR and the suppression rate of HGP obtained after insulin administration were increased by histidine administration (Fig. 2*B*). The suppression of hepatic gene expression of *Pck1* and *G6pc* by insulin was augmented by histidine (Fig. 2*C*), even though histidine did not affect plasma insulin levels in the clamp tests (Supplementary Fig. 1). While PEPCK protein expression did not change significantly during the 120-min hyperinsulinemic-euglycemic clamping period, insulin administration reduced the levels of G6Pase protein, and this reduction was

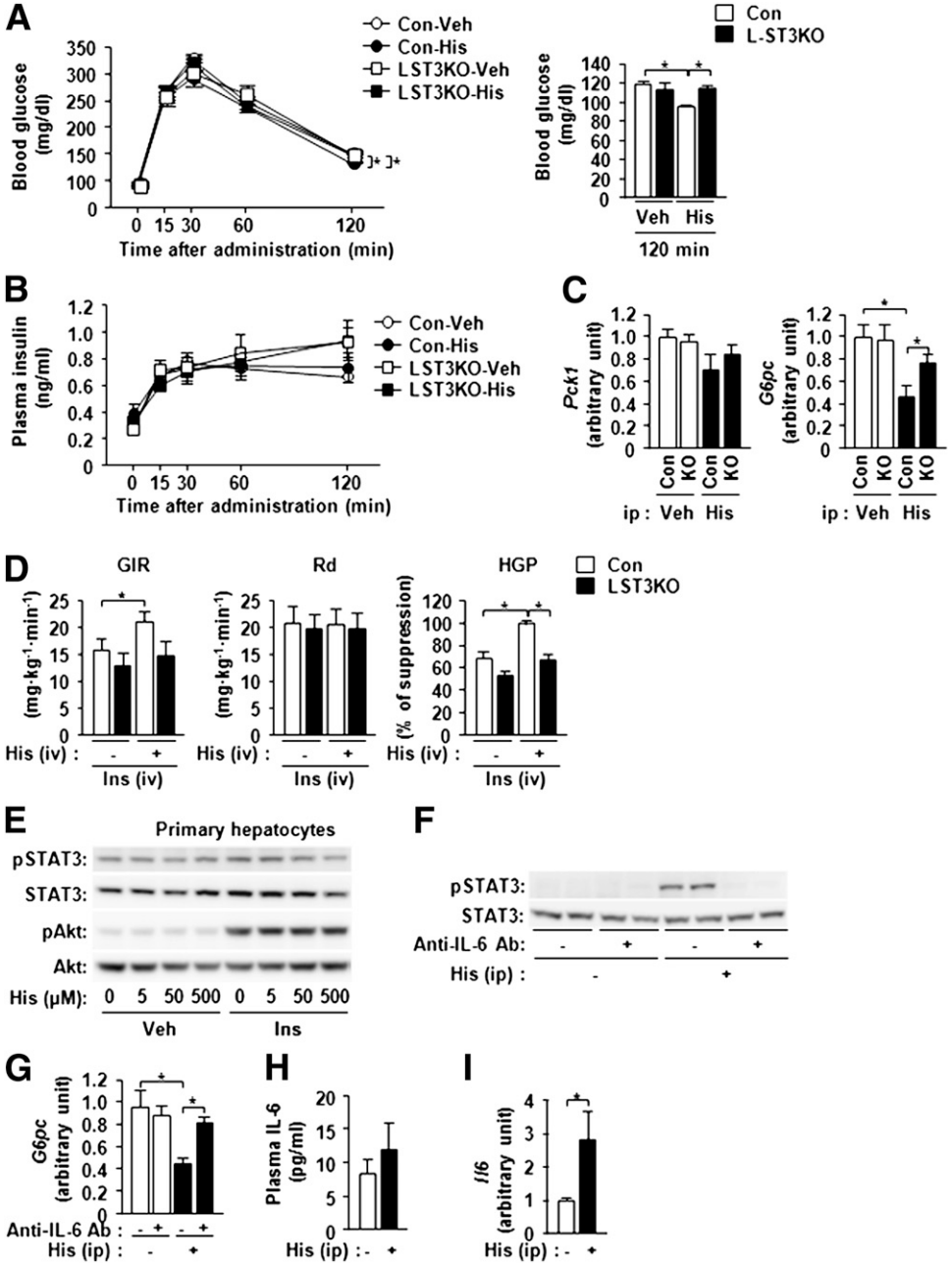

FIG. 3. Activation of hepatic IL-6/STAT3 signaling pathways is required for the histidine-mediated regulation of HGP. *A* and *B*: A glucose tolerance test was conducted after the intraperitoneal injection of histidine (His) in liver-specific STAT3-deficient (LST3KO) mice and controls (Con). Changes in blood glucose levels (*A*, *left panel*), blood glucose levels at 120 min after glucose loading (*A*, *right panel*), and changes in plasma insulin levels (*B*) are shown. *$P < 0.05$ ($n = 10$). *C*: Expression of hepatic *Pck1* and *G6pc* genes was analyzed by quantitative PCR at 120 min after the intraperitoneal injection of His into LST3KO and control (Con) mice. *$P < 0.05$ ($n = 6$–8). *D*: Hyperinsulinemic-euglycemic clamping was conducted as described in Fig. 2*A*. The GIR (*left panel*), R_d (*center panel*), and suppression (%) of HGP (*right panel*) were measured. *$P < 0.05$ ($n = 6$). *E*: Primary hepatocytes were treated with the different concentrations of histidine and insulin (Ins) shown in the figure, and the phosphorylation of STAT3, Akt, p70 S6 kinase, and S6 ribosomal protein was analyzed. *F–I*: The effect of intraperitoneal histidine injection on liver function was investigated using an IL-6 neutralizing antibody. *F*: Phosphorylation of hepatic STAT3 was analyzed by Western blotting at 120 min after histidine administration. *G–I*: Hepatic *G6pc* gene expression (*G*), blood IL-6 levels (*H*), and hepatic *Il6* gene expression (*I*) at 120 min after histidine administration are shown. *$P < 0.05$ ($n = 7$–9).

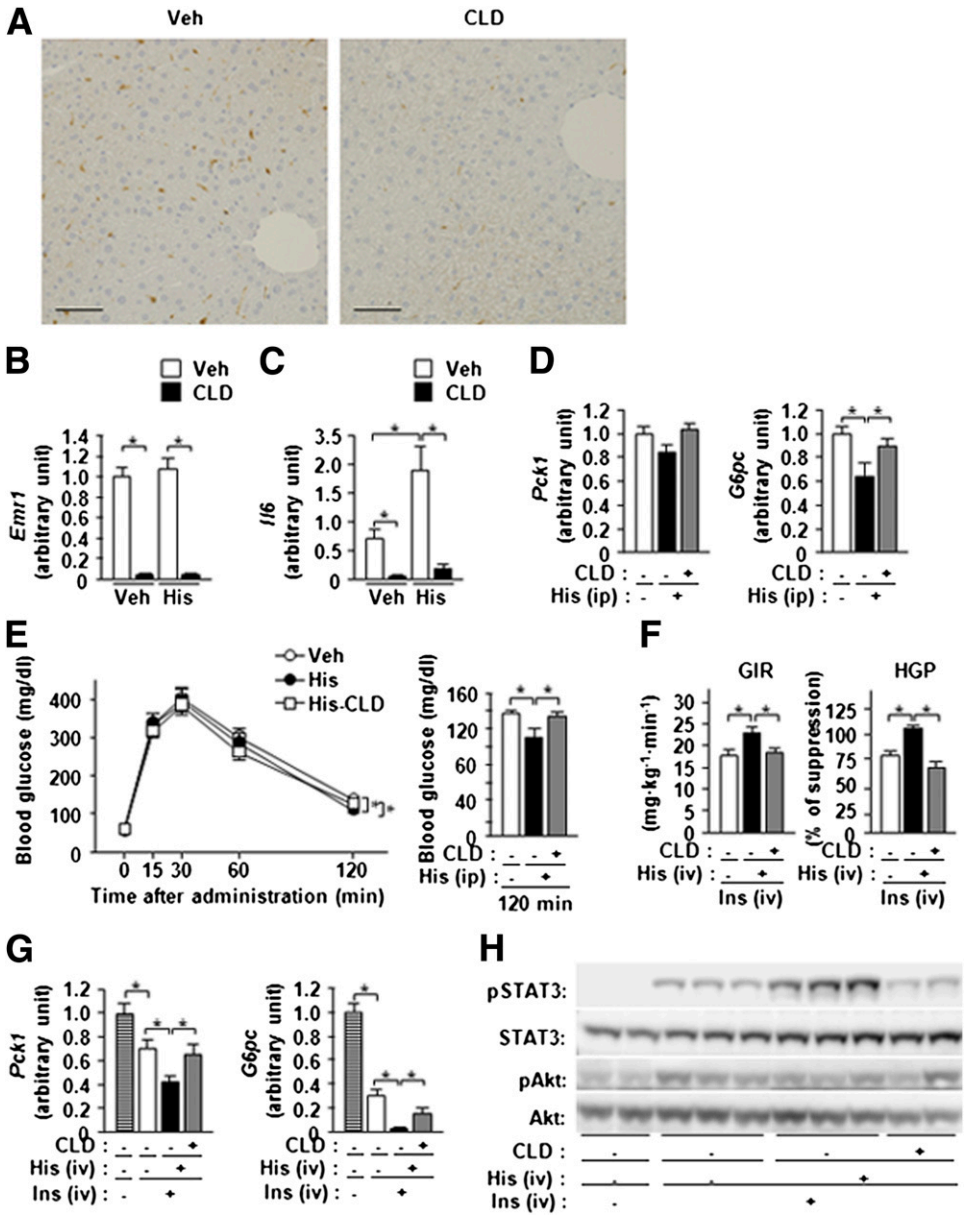

FIG. 4. Liver Kupffer cells are essential for the regulation of HGP by histidine (His). ***A*****: Immunostaining with an anti-Mac-2 antibody was performed in Kupffer cells after the administration of CLD. Scale bars, 50 nm.** ***B–D*****: Histidine was injected intraperitoneally into mice pretreated with CLD, and** ***Emr1*** **(*B*),** ***Il6*** **(*C*), and** ***Pck1*** **and** ***G6pc*** **(*D*) gene expression in the liver was quantitated using PCR at 120 min after histidine administration. *$P < 0.05$ ($n = 5–7$).** ***E*****: The intraperitoneal injection of histidine and a glucose tolerance test were conducted in mice pretreated with CLD. Changes in blood glucose levels (*E*,** ***left panel*****) and blood glucose levels at 120 min after glucose loading (*E*,** ***right panel*****) are shown. *$P < 0.05$ ($n = 9$).** ***F–H*****: With the continuous administration of histidine, hyperinsulinemic-euglycemic clamping (Fig. 2*A*) was performed in mice pretreated with CLD, and the GIR (*left panel*) and suppression (%) of HGP (*right panel*) were measured. In addition, the expression of hepatic** ***Pck1*** **and** ***G6pc*** **genes (*G*) and the phosphorylation of hepatic STAT3 and Akt (*H*) at 120 min after insulin (Ins) administration were examined. *$P < 0.05$ ($n = 6$).**

augmented by histidine administration (Fig. 2*D*). Phosphorylation of STAT3, but not Akt, was observed at 120 min after insulin administration (Fig. 2*E*), and histidine enhanced the phosphorylation of STAT3 caused by hyperinsulinemia (Fig. 2*E*). To evaluate the histidine effect of HGP with fasting levels of insulin, we performed euglycemic-pancreatic clamp tests (Fig. 2*F*). No significant changes in GIR or HGP resulted from the presence or absence of histidine injection (Fig. 2*G*).

Activation of hepatic IL-6/ STAT3 signaling pathways essential for the histidine-mediated regulation of hepatic glucose metabolism. We investigated the significance of hepatic STAT3 in the suppression of HGP after histidine administration using 8- to 10-week-old LST3KO mice, which had not yet developed insulin resistance (5). In a glucose tolerance test with histidine administration, blood glucose levels were significantly decreased in the control, but not LST3KO, mice at 120 min after glucose loading (Fig. 3*A*), even though no significant difference in plasma insulin levels was observed between the groups (Fig. 3*B*). While *G6pc* gene expression was downregulated at 120 min after the intraperitoneal administration of histidine in the control mice, no such downregulation was observed in the LST3KO mice (Fig. 3*C*). In the hyperinsulinemic-euglycemic clamp tests, histidine increased the GIR and the suppression of HGP caused by insulin administration

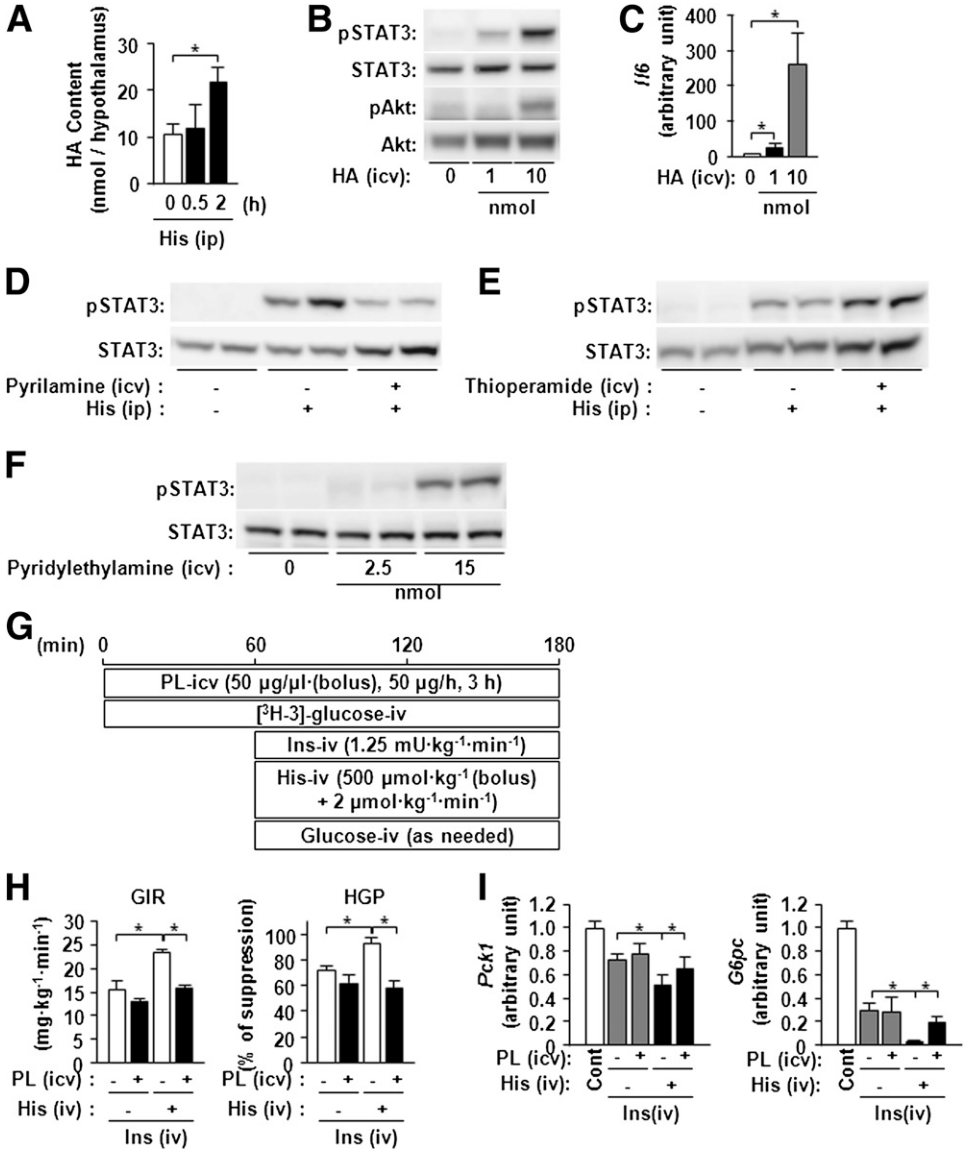

FIG. 5. Central histamine action is required for the regulation of HGP by histidine. *A*: Hypothalamic histamine (HA) content was measured at 30 and 120 min after the intraperitoneal administration of histidine (His). *$P < 0.05$ ($n = 5$). *B* and *C*: HA was injected into the ventricle at the concentrations shown in the figure, and the phosphorylation of hepatic STAT3 and Akt (*B*) and expression of hepatic *Il6* gene (*C*) were analyzed at 180 min postadministration. *$P < 0.05$ ($n = 5$). *D*: Histidine was injected intraperitoneally at 15 min after the lateral ventricular administration of the histamine H_1 receptor antagonist pyrilamine (50 μg/mouse), and phosphorylation of hepatic STAT3 was analyzed at 120 min after histidine administration. *E*: The intraperitoneal administration of histidine was performed at 30 min after the lateral ventricular administration of the histamine H_3 receptor antagonist thioperamide (50 nmol/mouse), and phosphorylation of hepatic STAT3 was analyzed at 120 min after histidine administration. *F*: Phosphorylation of hepatic STAT3 was analyzed ($n = 4$) at 180 min after the lateral ventricular administration of the histamine H_1 receptor agonist pyridylethylamine at the concentrations indicated in the figure. *G–I*: A hyperinsulinemic-euglycemic clamp (Ins injection) was performed with the continuous administration of histidine (His) or saline (-) in the jugular vein and pyrilamine (PL) or artificial cerebrospinal fluid (-) in the lateral ventricle, as shown in the schematic (*G*). The GIR (*H*, *left panel*) and the suppression (%) of HGP (*H*, *right panel*) are shown. ANOVA for GIR (*H*, *left panel*) revealed a significant effect of pyrilamine and histidine, as well as an interaction between pyrilamine and histidine ($P < 0.05$, $n = 6$). ANOVA for HGP (*H*, *right panel*) revealed a significant main effect of pyrilamine ($P < 0.05$) and an interaction between pyrilamine and histidine ($P < 0.01$, $n = 6$). In addition, the expression of hepatic *Pck1* and *G6pc* genes was analyzed at 120 min after insulin administration (*I*). *$P < 0.05$ ($n = 6$). Cont, control.

in the control animals but not in the LST3KO mice (Fig. 3*D*). Insulin activates hepatic STAT3 via the central nervous system and the subsequent upregulation of *Il6* gene expression in the liver (9). Indeed, not only insulin but also histidine failed to activate STAT3 in primary hepatocytes (Fig. 3*E*). Histidine also had no effect on the Akt and S6 kinase pathway (Fig. 3*E*). Therefore, we used an IL-6 neutralizing antibody to investigate the role of IL-6 in the histidine-mediated activation of hepatic STAT3. The administration of the anti–IL-6 antibody prior to histidine administration attenuated the histidine-induced phosphorylation of STAT3 (Fig. 3*F*) as well as the suppression of *G6pc* gene expression (Fig. 3*G*). Histidine administration did not significantly change the plasma levels of IL-6 (Fig. 3*H*) but significantly upregulated *Il6* gene expression in the liver (Fig. 3*I*). These results suggest that, similar to insulin, histidine activates hepatic STAT3 through the upregulation of hepatic IL-6.

Kupffer cells are essential for the histidine-mediated regulation of hepatic glucose metabolism. In the liver, Kupffer cells play an important role in the production of

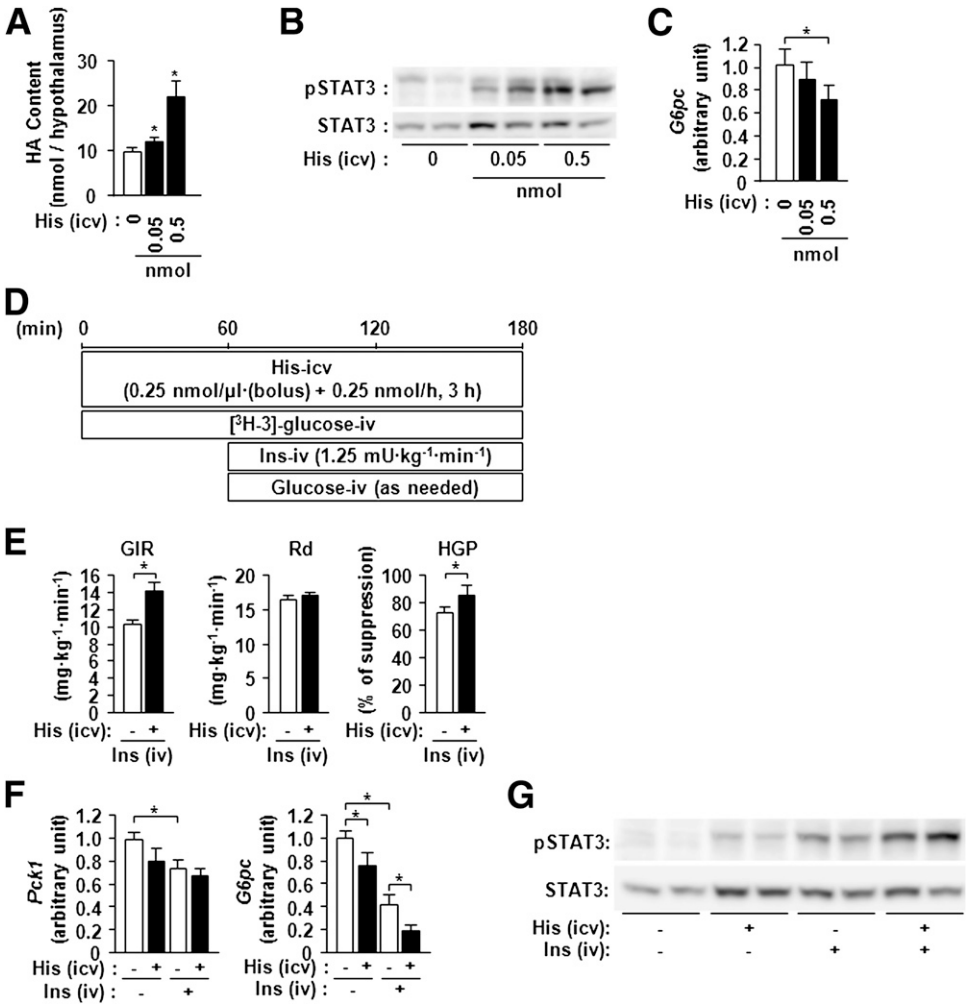

FIG. 6. ICV administration of histidine (His) augments the insulin-dependent suppression of HGP. *A–C*: With use of the doses indicated in the figure, histidine was injected rapidly into the lateral ventricle, and hypothalamic histamine (HA) content (*A*), phosphorylation of hepatic STAT3 (*B*), and expression of the hepatic *G6pc* gene (*C*) were analyzed at 180 min postadministration. *$P < 0.05$ ($n = 5–8$). *D–G*: As shown in the schematic (*D*), hyperinsulinemic-euglycemic clamping was performed concurrently with the continuous administration of histidine or artificial cerebrospinal fluid (-) into the lateral ventricle. The GIR (*E*, *left panel*), R_d (*E*, *center panel*), and suppression (%) of HGP (*E*, *right panel*) were measured. *$P < 0.05$ ($n = 6$). In addition, the expression of hepatic *Pck1* and *G6pc* genes (*F*) and phosphorylation of hepatic STAT3 (*G*) were analyzed at 120 min after insulin administration. ANOVA for *Pck1* (*F*, *left panel*) revealed a significant main effect of Ins ($P < 0.05$) but no significant interaction between Ins and His ($P = 0.060$, $n = 6$). ANOVA for *G6pc* (*F*, *right panel*) revealed a significant main effect of Ins and histidine ($P < 0.05$), but no significant interaction between insulin and histidine treatments ($P = 0.120$, $n = 6$).

IL-6 (22,23); therefore, we depleted Kupffer cells by the intravenous injection of CLD and investigated the function of histidine (22). CLD administration resulted in the disappearance of Mac-2–positive cells (Fig. 4*A*) and significantly downregulated the expression of the *Emr1* gene (Fig. 4*B*), which is specific to Kupffer cells in the liver. In addition, the depletion of Kupffer cells diminished the expression of hepatic *Il6* (Fig. 4*C*). Histidine administration, which significantly downregulates the expression of the hepatic *G6pc* gene, did not change its expression after CLD administration (Fig. 4*D*). In the glucose tolerance test, histidine administration significantly reduced blood glucose levels at 120 min after glucose loading; however, this reduction was diminished after CLD administration (Fig. 4*E*). In a hyperinsulinemic-euglycemic clamp experiment, histidine augmented the effects of insulin on *Pck1* and *G6pc* gene expression, HGP, and the GIR, but the augmentation of insulin action by histidine was diminished by CLD administration (Fig. 4*F* and *G*). Although histidine augmented the STAT3 phosphorylation caused by hyperinsulinemia, this augmentation was attenuated by CLD administration (Fig. 4*H*). These results indicate that hepatic IL-6 expression in Kupffer cells is essential for the histidine-mediated regulation of hepatic glucose metabolism.

Central histamine mediates the regulation of hepatic glucose metabolism by histidine. Histidine is a precursor of histamine; therefore, the levels of histamine in the hypothalamus are reportedly increased after the systemic administration of histidine (24,25). Thus, we investigated the involvement of central histamine in the histidine-mediated activation of hepatic STAT3 and regulation of HGP. As reported previously, histidine administration increased the levels of histamine in the hypothalamus (Fig. 5*A*). In addition, the ICV administration of histamine induced the phosphorylation of hepatic STAT3 (Fig. 5*B*) and upregulated the expression of *Il6* in the liver (Fig. 5*C*). The lateral ventricular administration of the histamine H_1 receptor antagonist pyrilamine prior to systemic histidine administration attenuated the phosphorylation of hepatic STAT3 (Fig. 5*D*), whereas the ICV administration of the histamine

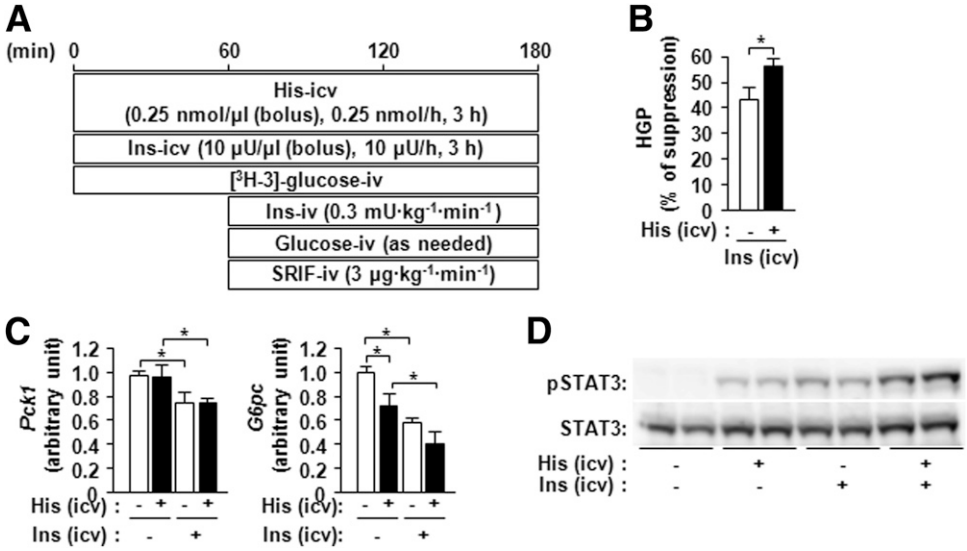

FIG. 7. Central histidine (His) action augments the suppression of HGP by insulin ICV administration. *A–D*: As shown in the schematic (*A*), continuous lateral ventricular injection of histidine, artificial cerebrospinal fluid (aCSF) (-), and insulin (Ins) in pancreatic clamping was performed. The suppression (%) of HGP, **P* < 0.05 (*n* = 6) (*B*), expression of hepatic *Pck1* and *G6pc* genes at 180 min after the start of lateral ventricular administration (*C*), and phosphorylation of hepatic STAT3 (*D*) were analyzed. ANOVA for *Pck1* expression (*C*, *left panel*) revealed a significant main effect of Ins (*P* < 0.05) but no significant interaction between insulin and histidine (*P* = 0.964, *n* = 6). ANOVA for *G6pc* expression (*C*, *right panel*) revealed a significant main effect of insulin and histidine (*P* < 0.05) but no significant interaction between insulin and histidine treatments (*P* = 0.116, *n* = 6).

H3 receptor antagonist thioperamide increased STAT3 phosphorylation (Fig. 5*E*). Conversely, the ICV administration of the histamine H_1 receptor agonist pyridylethylamine activated hepatic STAT3 (Fig. 5*F*). To elucidate the role of central histamine/histamine H_1 receptor in the histidine-mediated regulation of HGP, we performed a hyperinsulinemic-euglycemic clamp study with the concurrent ICV administration of pyrilamine and intravenous administration of histidine (Fig. 5*G*). Although histidine administration augmented the action of insulin on *Pck1* and *G6pc* gene expression, HGP, and the GIR, the ICV administration of pyrilamine blocked this augmentation (Fig. 5*H* and *I*).

ICV administration of histidine augments the insulin-dependent suppression of HGP. We administered histidine into the ventricle to investigate the involvement of the central-mediated regulation of HGP by histidine. As with intraperitoneal injection, the ICV administration of histidine increased the hypothalamic histamine content (Fig. 6*A*). In addition, histidine ICV administration upregulated the phosphorylation of hepatic STAT3 (Fig. 6*B*) and suppressed *G6pc* gene expression in the liver (Fig. 6*C*). We then investigated the effect of histidine ICV administration on the insulin-dependent suppression of HGP (Fig. 6*D*). In hyperinsulinemic-euglycemic clamping, the ICV administration of histidine increased the GIR and augmented the suppression of HGP (Fig. 6*E*). The ICV administration of histidine suppressed the expression of the *G6pc* gene and augmented the suppression of gene expression by the systemic administration of insulin (Fig. 6*F*), whereas *Pck1* gene expression tended to decrease, albeit insignificantly, after histidine administration (Fig. 6*F*). Furthermore, histidine ICV administration increased the phosphorylation of hepatic STAT3 and additively enhanced the hepatic phosphorylation of STAT3 associated with hyperinsulinemia (Fig. 6*G*). These results indicate that the suppression of HGP by the systemic administration of insulin is augmented by the central administration of histidine.

We then injected insulin and histidine into the ventricle to investigate the effect of central histidine on the regulation of HGP by central insulin action (Fig. 7*A*). The ICV administration of insulin was performed with the concurrent administration of somatostatin to block the secretion of endogenous insulin caused by insulin ICV administration (Fig. 7*A*). As reported previously, the ICV administration of insulin suppressed HGP, while the ICV administration of histidine augmented this suppression (Fig. 7*B*). In addition, with the ICV administration of insulin the expression of the *Pck1* gene decreased, albeit insignificantly, whereas the expression of the *G6pc* gene was significantly downregulated (Fig. 7*C*). Histidine administration augmented the suppression of *G6pc* gene expression (Fig. 7*C*). Hepatic STAT3 was phosphorylated after the ICV administration of insulin or histidine, and their coadministration enhanced its phosphorylation (Fig. 7*D*). These results indicate that the central administration of histidine augments the suppression of HGP by central insulin action.

Central histidine action is independent of central insulin action. Next, we investigated the cross-talk between central histidine and central insulin action, both of which activate hepatic STAT3. As pyrilamine administration inhibited hepatic STAT3 phosphorylation mediated by the systemic administration of histidine (Fig. 5*D*), the phosphorylation of hepatic STAT3 was attenuated by the administration of pyrilamine prior to the ICV administration of histidine (Fig. 8*A*). Conversely, the phosphorylation of hepatic STAT3 induced by the ICV administration of insulin was not altered by the preadministration of pyrilamine (Fig. 8*B*). Furthermore, in a study using Hrh1KO mice (16) the phosphorylation of hepatic STAT3 was significantly attenuated after the ICV administration of histidine (Fig. 8*C*) in the same manner as that after pyrilamine administration. However, no significant change in hepatic STAT3 phosphorylation was induced by the ICV administration of insulin to the Hrh1KO mice (Fig. 8*D*).

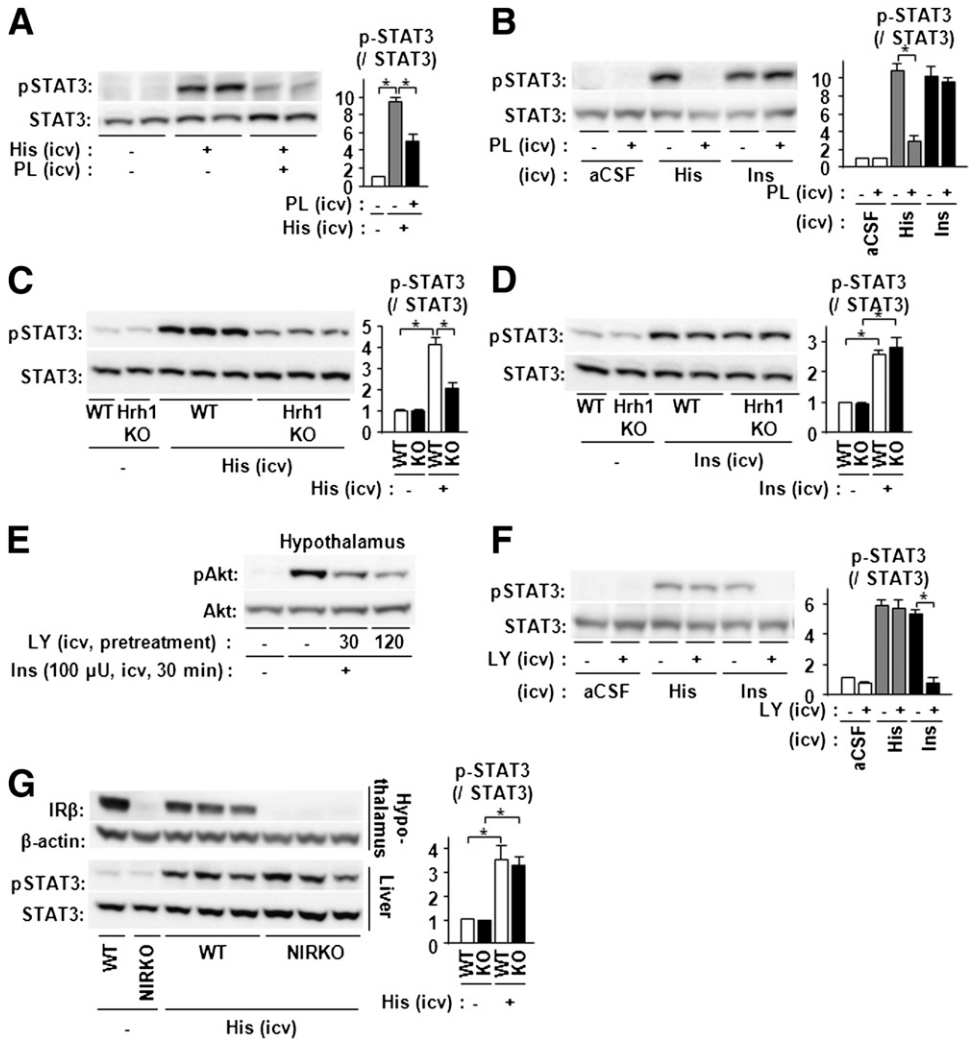

FIG. 8. Central histidine (His) action on the liver is independent of central insulin (Ins) action. *A* and *B*: Phosphorylation of STAT3 was analyzed at 180 min after the intravenous administration of somatostatin (3 μg · kg^{-1} · min^{-1}) and the lateral ventricular administration of histidine (*A* and *B*) or insulin (*B*), which was performed at 15 min after the ICV preadministration of the histamine H_1 antagonist pyrilamine (50 μg/mouse). Quantitation is represented as means ± SE (n = 6–8). *C* and *D*: Somatostatin administration (3 μg · kg^{-1} · min^{-1}) and the lateral ventricular administration of histidine (*C*) or insulin (*D*) were performed in Hrh1KO mice, and the phosphorylation of hepatic STAT3 was analyzed at 180 min postadministration. Quantitation is represented as means ± SE (n = 6). *E*: The ICV administration of insulin (100 μU/mouse, bolus) was performed at 30 and 120 min after the administration of the PI3-K inhibitor LY294002 (10 nmol/mouse; LY). The phosphorylation of hypothalamic Akt at 30 min after insulin administration is shown. *F*: Somatostatin administration (3 μg · kg^{-1} · min^{-1}) and the lateral ventricular administration of histidine or insulin were performed at 120 min after the lateral ventricular administration of LY294002 (10 nmol/mouse; LY), and the phosphorylation of hepatic STAT3 was analyzed at 180 min postadministration. Quantitation is represented as means ± SE (n = 5). *G*: Somatostatin (3 μg · kg^{-1} · min^{-1}) and histidine were administered into NIRKO mice, and the levels of hypothalamic insulin receptor (IRβ), β-actin, and hepatic STAT3 were analyzed at 180 min postadministration. Quantitation is represented as means ± SE (n = 6). aCSF, artificial cerebrospinal fluid; WT, wild type.

Insulin-dependent phosphorylation of hypothalamic Akt was attenuated by the ICV administration of the PI3-K inhibitor LY294002 (Fig. 8*E*). We therefore investigated the effect of the ICV administration of histidine or insulin, with the concurrent administration of LY294002, on the phosphorylation of hepatic STAT3. LY294002 inhibited the insulin-induced phosphorylation of hepatic STAT3 but did not affect its phosphorylation by histidine (Fig. 8*F*). We also examined mice lacking central insulin receptors. In the NIRKO mice, as in the control mice, the ICV administration of histidine induced hepatic STAT3 phosphorylation (Fig. 8*G*). These results suggest that central histidine action mediated by the histamine H_1 receptor is independent of central insulin action but that they function additively to suppress HGP.

DISCUSSION

The central nervous system monitors hormonal and nutritional changes that regulate energy metabolism in peripheral tissues. In this study, we revealed that an increase in blood histidine levels acts on the central nervous system, activates hepatic IL-6/STAT3 signaling, suppresses the expression of gluconeogenic genes in the liver, and augments the insulin-dependent suppression of HGP. These findings suggest that histidine can be a target molecule for the treatment of type 2 diabetes. Recently, plasma histidine levels were reported to show a significant inverse association with fasting and 2-h blood glucose levels during glucose tolerance tests in individuals without diabetes or newly diagnosed male patients with type 2 diabetes (14). Histidine was also shown to improve the hyperglycemia induced by

the central administration of 2-deoxyglucose in rodents (26); however, the mechanism by which histidine lowers blood glucose levels is not clear. In the current study, the intraperitoneal administration of histidine induced the phosphorylation of hepatic STAT3 and reduced blood glucose levels associated with glucose loading. Consistent with a previous study showing that histidine is not involved in the secretion of insulin (27), histidine did not significantly affect plasma insulin levels in the current study. In the hyperinsulinemic-euglycemic clamp experiment, the intravenous administration of histidine increased GIR and the suppression of HGP. Such histidine action was not observed in LST3KO mice or Kupffer cell–depleted mice, suggesting that the activation of hepatic IL-6/STAT3 signaling plays an important role in the histidine-mediated suppression of HGP. The gene expression of *G6pc* and *Pck1* was downregulated by histidine administration in the hyperinsulinemic-euglycemic clamp experiment, with a clear reduction in the protein levels of G6Pase but not PEPCK. These findings suggest that the downregulation of G6Pase expression causes the suppression of HGP by histidine. However, the activation of hepatic STAT3 by histidine alone is relatively weak, resulting in the mild suppression of *G6pc* gene expression but not of *Pck1*. Furthermore, euglycemic-pancreatic clamp tests with histidine did not reveal a significant change in HGP. These findings may explain why histidine administration alone is not enough to affect the homeostasis of blood glucose levels.

In the brain, histidine is converted into histamine by histidine decarboxylase in the tuberomammillary nucleus of the posterior hypothalamus (24,25,28). Consistent with a previous study (25), hypothalamic histamine content was increased by histidine administration in the current study. A previous study using rats showed that enhanced central histamine action due to histidine administration suppresses food intake (25). In contrast, both Hrh1KO mice (29) and histidine decarboxylase knockout mice (30) present with insulin resistance as well as obesity. In addition, the development of obesity and diabetes caused by the antipsychotic drug olanzapine in humans is reportedly linked to inhibition of the histamine H_1 receptor (31). Such phenotypes may indicate that the action of histamine affects not only food intake but also glucose metabolism. In this study, the central administration of histamine H_1 receptor antagonist attenuated the activation of hepatic STAT3 and the suppression of HGP induced by the systemic administration of histidine. Furthermore, the central administration of histidine also activated hepatic STAT3 while suppressing HGP. These findings indicate that the histidine-induced suppression of HGP, as well as the suppression of food intake, is mediated by central histamine action. Because the histamine-containing neurons in the tuberomammillary nucleus project into most of the brain (28), the precise mechanism in the brain by which central histamine activates hepatic STAT3 remains unclear. This histidine action is additive to the insulin-induced suppression of HGP. The activation of hepatic STAT3 by central insulin action was not blocked by the central administration of a histamine H_1 receptor antagonist or in Hrh1KO mice. In contrast, the activation of hepatic STAT3 by central histidine was not blocked by the central administration of a PI3-K inhibitor or in NIRKO mice, suggesting that the mechanism underlying the action of central histidine is independent of insulin action.

This study demonstrates that the activation of hepatic IL-6/STAT3 signaling pathways and downregulation of hepatic gluconeogenic gene expression by histidine are mediated by central histamine action involving the histamine H_1 receptor. Central histidine action, which has a central nervous system mechanism that is independent of insulin, augments the insulin-dependent suppression of glucose production in the liver. This study indicates that the central mechanism by which histidine regulates hepatic glucose metabolism and activates hepatic STAT3 is a potential target for the treatment of type 2 diabetic patients with elevated gluconeogenesis.

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