

Interaction Between the Central and Peripheral Effects of Insulin in Controlling Hepatic Glucose Metabolism in the Conscious Dog

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The importance of hypothalamic insulin action to the regulation of hepatic glucose metabolism in the presence of a normal liver/brain insulin ratio (3:1) is unknown. Thus, we assessed the role of central insulin action in the response of the liver to normal physiologic hyperinsulinemia over 4 h. Using a pancreatic clamp, hepatic portal vein insulin delivery was increased three- or eightfold in the conscious dog. Insulin action was studied in the presence or absence of intracerebroventricularly mediated blockade of hypothalamic insulin action. Euglycemia was maintained, and glucagon was clamped at basal. Both the molecular and metabolic aspects of insulin action were assessed. Blockade of hypothalamic insulin signaling did not alter the insulin-mediated suppression of hepatic gluconeogenic gene transcription but blunted the induction of glucokinase gene transcription and completely blocked the inhibition of glycogen synthase kinase-3 β gene transcription. Thus, central and peripheral insulin action combined to control some, but not other, hepatic enzyme programs. Nevertheless, inhibition of hypothalamic insulin action did not alter the effects of the hormone on hepatic glucose flux (production or uptake). These data indicate that brain insulin action is not a determinant of the rapid (<4 h) inhibition of hepatic glucose metabolism caused by normal physiologic hyperinsulinemia in this large animal model. *Diabetes* 62:74–84, 2013

The relevance of hypothalamic insulin action to insulin's acute inhibitory effect on hepatic glucose metabolism is unclear. In rodents, hypothalamic insulin action can suppress endogenous glucose production (EGP) (1–11). The effect has been attributed to increased hypothalamic phosphatidylinositol 3-kinase (PI3K) activity, a neurally driven phosphorylation of hepatic signal transducer and activator of transcription 3 (STAT3) (3,4), the suppression of gluconeogenic gene expression (3,5,10,11), and consequent inhibition of gluconeogenesis (11).

We recently confirmed that the insulin–brain–liver signaling axis established in rodents (involving increased hypothalamic phospho [P]-Akt and hepatic P-STAT3 and

reduced hepatic gluconeogenic mRNA expression) is conserved in the dog (12). Moreover, selective brain hyperinsulinemia, brought about in the presence of basal hepatic insulin levels, also regulated glycogen synthase kinase-3 β (GSK3 β) and glucokinase (GK) gene expression. Unlike in rodents, however, brain insulin action did not alter EGP or gluconeogenesis, but instead increased hepatic glucose uptake. This was explained by an increase in net hepatic glycogen synthesis associated with reductions in GSK3 β mRNA and protein expression and a decrease in glycogen synthase (GS) phosphorylation by the fourth hour of brain hyperinsulinemia.

Endogenous insulin, secreted into the portal vein, is subject to considerable hepatic extraction, resulting in an approximately threefold gradient at the liver compared with nonhepatic tissues (including the brain) (13,14). In the rodent (1–11) and dog (12) experiments cited above, the effect of brain hyperinsulinemia was studied during clamps in which insulin was infused into a peripheral vessel rather than the portal vein, thereby disrupting the normal physiologic insulin gradient. Thus, the ability of hypothalamic insulin action to regulate EGP has only been defined in the presence of relative hepatic insulin deficiency (i.e., the level of insulin at the liver was no greater than at the brain). It is possible that the peripheral effects of the hormone may complement or mask its central nervous system effects on liver glucose metabolism. It is currently unknown how insulin's central and peripheral effects interact to control hepatic glucose metabolism. Thus, the aim of this study was to determine whether brain insulin action impacts the regulation of key hepatic glucoregulatory enzymes and hepatic glucose metabolism during a physiologic rise in insulin (in the presence of a normal 3:1 liver/brain insulin gradient) in the conscious dog.

RESEARCH DESIGN AND METHODS

Animal care and surgical procedures. Experiments were performed on conscious dogs of either sex (19–24 kg). The surgical facility met the American Association for the Accreditation of Laboratory Animal Care standards, and the protocol was approved by the Institutional Animal Care and Use Committee.

Seventeen days prior to study, the jejunal and splenic veins (infusion), femoral artery, hepatic portal vein, and hepatic vein (sampling) were catheterized, and ultrasonic flow probes were placed around the hepatic artery and portal vein, as described previously (15). Ten days prior to study, infusion catheters were placed bilaterally in the carotid and vertebral arteries, a sampling catheter was inserted in the jugular vein, and intracerebroventricular (ICV) cannulation was performed stereotaxically as described previously (12). All dogs were healthy, as indicated by a return to presurgical food intake and body weight, leukocyte count <18,000/mm³, hematocrit >35%, and normal stools.

Experimental design. Experiments consisted of equilibration (–180 to –90 min), basal (–90 to 0 min), and experimental (0–240 min) periods. At –180 min,

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salinized $^2\text{H}_2\text{O}$ (3 mL/kg) was administered intravenously. At the same time, [^3H]glucose (35 μCi priming dose; 0.35 $\mu\text{Ci}/\text{min}$ constant infusion) was started. A pancreatic clamp was initiated at -180 min with peripheral somatostatin (0.8 $\mu\text{g}/\text{kg}/\text{min}$) and basal portal infusion of glucagon (0.57 ng/kg/min). Insulin infusion was matched to the animal's endogenous rate of secretion by adjusting its intraportal insulin infusion rate to maintain euglycemia, with the last change being made at least 30 min before the start of the basal period.

In the first experiments (18-h fast), at -60 min, ICV infusion of artificial cerebrospinal fluid (aCSF; $n = 5$), the ATP-sensitive K^+ channel (K_{ATP}) inhibitor glibenclamide (GLIB; $n = 5$), or the PI3K inhibitor LY294002 (LY; $n = 5$) was initiated using rates previously described (8,12). At 0 min, the portal vein insulin infusion was increased to 750 $\mu\text{U}/\text{kg}/\text{min}$ to bring about a physiologic threefold rise in systemic insulin. In control experiments (BASAL INS; no ICV infusion; $n = 7$), the basal portal vein insulin infusion rate remained unchanged.

In the second experiments (42-h fast), only LY was used to block hypothalamic insulin signaling, and the portal vein insulin infusion was increased to 1,800 $\mu\text{U}/\text{kg}/\text{min}$ at 0 min in order to bring about a physiologic eightfold increase in systemic insulin. ICV LY ($n = 6$) or aCSF ($n = 5$) infusion began at -60 min. The changes in study design were implemented in order to increase the likelihood that brain insulin action would produce a metabolic effect (higher brain insulin levels and greater potential for both suppression of gluconeogenesis and stimulation of glycogen synthesis following a longer fast). It should be noted that a 42-h fast in the dog does not induce hypoglycemia, raise the plasma levels of stress hormones, or deplete liver glycogen (16,17). In control experiments, the basal insulin infusion rate was not changed (BASAL INS+aCSF, $n = 4$; BASAL INS+LY, $n = 4$). As previously observed (8,12), LY infusion (in the presence of basal insulin) did not alter any signaling or metabolic parameter. Thus, the data from these two groups, which were presented separately in our previous paper (12), were pooled and are presented in this study in combination (BASAL INS; $n = 8$). In additional experimental subsets, LY ($n = 3$) or aCSF ($n = 3$) were infused, and insulin was elevated eightfold as described above; however, the experiments were terminated at 60 min to assess the rapidity of brain insulin action and blockade.

In the final experiments (42-h fast), at 0 min, the portal vein insulin infusion was increased to 495 $\mu\text{U}/\text{kg}/\text{min}$, and insulin was infused into the head arteries (362 $\mu\text{U}/\text{kg}/\text{min}$). This brought about the same level of insulin at the liver and brain. ICV LY ($n = 5$) or aCSF ($n = 6$) infusion began at -60 min. A decrease in arterial nonesterified fatty acid (NEFA) levels (due to mild arterial hyperinsulinemia) was prevented by peripheral infusion of intralipid plus heparin (18). These changes limited peripheral insulin action, thereby increasing the potential for observing a central effect of insulin on hepatic glucose metabolism. These animals were compared with the control group (BASAL INS; $n = 8$) described above.

In all studies, glucose was infused to maintain euglycemia. Immediately after obtaining the final blood sample, the animals were killed, and liver tissue was freeze-clamped and stored at -70°C . Immediately thereafter, the cranial vault was opened, and hypothalamic tissue was excised and freeze-clamped as described elsewhere (12).

Nuclear magnetic resonance (^2H) spectroscopy. Whole-body gluconeogenesis and glycogenolysis were calculated using the $^2\text{H}_2\text{O}$ analysis–nuclear magnetic resonance (NMR) detection method of Burgess et al. (19) as adapted in our laboratory (15).

Biochemical analysis. Protein and mRNA levels were assayed using standard Western blotting and real-time PCR procedures as described previously (15). The figures depict averages obtained from analysis of biopsies from the three largest liver lobes.

Metabolic analysis. We assessed glucose flux by measuring net hepatic glucose balance (NHGB; the sum of hepatic glucose output and uptake) and its components: the net rate of glucose-6-phosphate formation from the glycolytic/gluconeogenic and glycogen synthetic/glycogenolytic pathways as described previously (20). [^3H]glucose and $^2\text{H}_2\text{O}$ were used to determine EGP and its components, gluconeogenesis and glycogenolysis, as described previously (15). Hormone and substrate concentrations were determined using standard procedures (15).

Statistical analysis. Metabolic data were analyzed using two-way repeated-measures ANOVA (group \times time) (SigmaStat, SPSS Inc.). Molecular data were analyzed using one-way ANOVA. Significance was defined as $P < 0.05$.

RESULTS

The effect of brain insulin action on the liver in the presence of a modest (threefold) increase in insulin release. In the first set of experiments, arterial and hepatic insulin levels increased threefold, glucagon levels were kept basal, and arterial NEFA levels decreased by

50% in the three hyperinsulinemic groups (Fig. 1A–D). Arterial cortisol and catecholamine levels were basal and did not differ between groups (not shown). The glucose infusion rate required to maintain euglycemia (~ 2 mg/kg/min; Fig. 1E and F) and was not reduced by blockade of brain insulin action.

By the end of the experiment, hyperinsulinemia reduced EGP by $\sim 50\%$ in the aCSF group (Fig. 1G) and almost completely suppressed NHGB (Fig. 1H) due to reduced net hepatic glycogenolytic flux (Fig. 1I). There were no alterations in net hepatic gluconeogenic flux (Fig. 1J). In contrast, there were similar reductions in gluconeogenesis and glycogenolysis (Supplementary Fig. 1). The blockade of hypothalamic insulin action with ICV infusion of GLIB or LY had no effect on insulin-mediated suppression of NHGB, EGP, or any measured parameter relevant to glucose metabolism (Fig. 1E–J).

At the molecular level, the insulin–brain–liver axis (hypothalamic P-Akt and hepatic P-STAT3; Fig. 2A) and the hepatic insulin-signaling cascade (hepatic P-Akt and P-FOXO1; Fig. 2B) were activated in the aCSF group. Likewise, hyperinsulinemia suppressed gluconeogenic mRNA expression (Fig. 2C) without altering gluconeogenic enzyme levels (Fig. 2D). It also increased GK mRNA and protein levels (threefold and 35%, respectively; Fig. 2E), increased P-GSK3 β (50%; Fig. 2F), and reduced GS phosphorylation (30%; Fig. 2G). Blockade of hypothalamic insulin signaling (evident from the reduced hypothalamic P-Akt and/or hepatic P-STAT3 in the GLIB and LY groups; Fig. 2A) did not alter insulin's direct effects on hepatic insulin signaling or downstream glucoregulatory signals (Fig. 2B–G). Because the brain K_{ATP} channel is downstream of PI3K, GLIB blocked hepatic STAT3 phosphorylation without affecting hypothalamic Akt phosphorylation, confirming the involvement of K_{ATP} channels (11). The lack of increase in liver P-STAT3 demonstrates ICV GLIB and LY reached the hypothalamic neurons relevant to insulin action.

These data indicate that even though a small (threefold) rise in circulating insulin was sensed in the hypothalamus, the resulting change in neural input to the liver had no effect on the acute regulation of hepatic glucoregulatory gene expression nor any limiting effect on the reduction in EGP or net hepatic glucose balance.

The effect of brain insulin action on the liver in the presence of a larger (eightfold) increase in insulin release. In a second set of experiments, we evaluated the relevance of brain insulin action during a larger, but still physiologic (in regards to both dose and site of administration), hyperinsulinemia. Insulin increased \sim eightfold in the arterial, hepatic, and jugular vein plasma (Fig. 3A–C), whereas glucagon concentrations were clamped at basal (Fig. 3D), and circulating NEFA levels fell markedly ($\sim 95\%$; Fig. 3E). Arterial cortisol and catecholamine levels were basal and did not differ between groups (not shown). Euglycemia was maintained (Fig. 3F) using similar glucose infusion rates in both hyperinsulinemic groups (~ 15 mg/kg/min; Fig. 3G).

Hyperinsulinemia in aCSF-treated animals caused a rapid fall in EGP, eventually resulting in complete suppression of hepatic glucose production (Fig. 3H) and its component parts, gluconeogenesis and glycogenolysis (Supplementary Fig. 2). In contrast, the reduction of NHGB (Fig. 3I) was largely a function of a marked and sustained inhibition of net hepatic glycogenolytic flux (Fig. 3J). Net hepatic gluconeogenic flux was transiently decreased (Fig. 3K) in

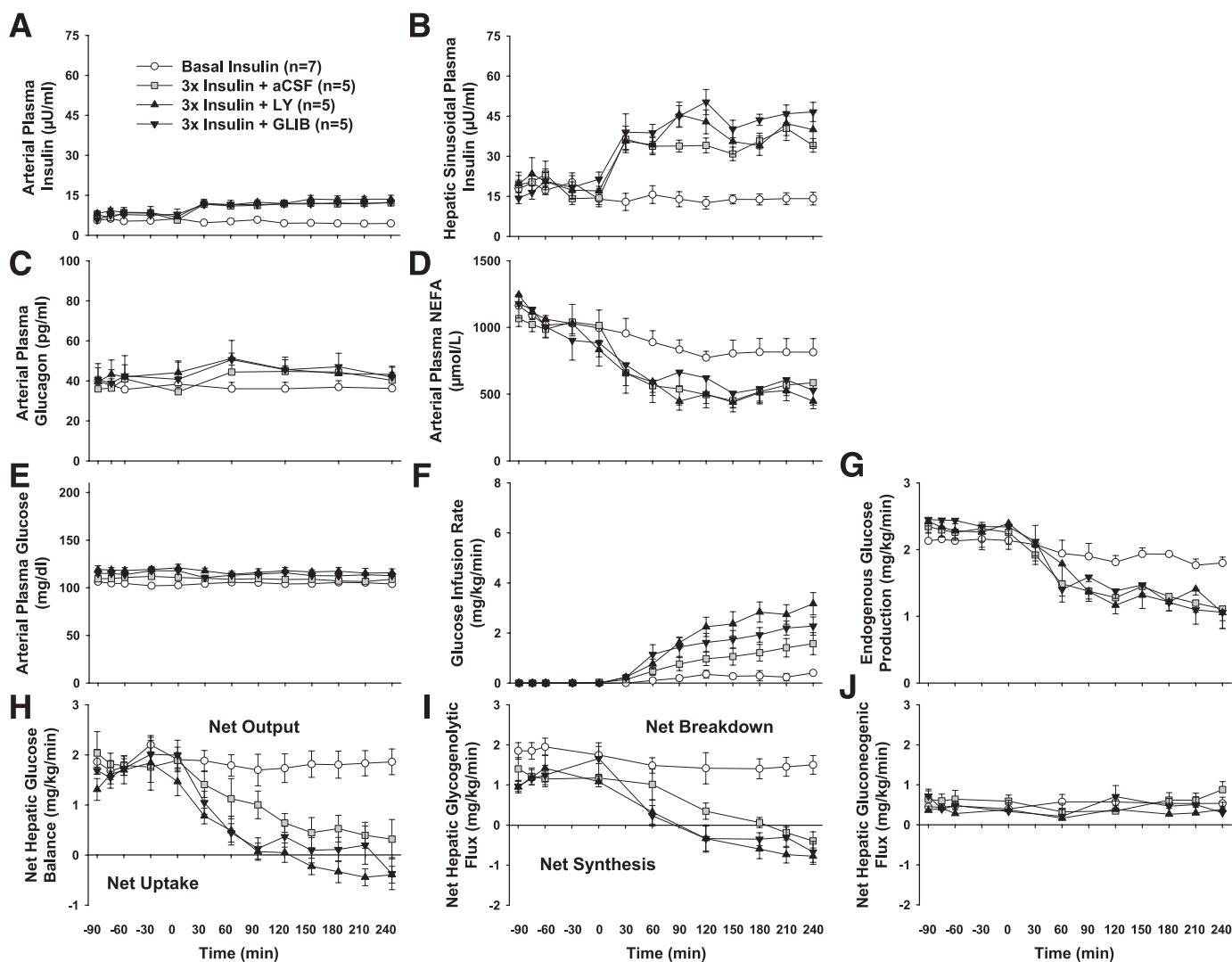


FIG. 1. Metabolic response to a threefold increase in portal vein insulin infusion in dogs maintained on a pancreatic clamp (compared with basal insulin-infused controls). *A–D*: Circulating insulin, glucagon, and NEFA levels. *E–J*: Glucose flux parameters. Euglycemia was maintained (*E*) with similar rates of glucose infusion (*F*) between groups. Threefold insulin caused the suppression of EGP (*G*) and NHGB (*H*). The alteration in NHGB was a function of suppressed net hepatic glycogenolytic flux (*I*) with no effect on net hepatic gluconeogenic flux (*J*). Values are means \pm SEM; $n = 5$ in each hyperinsulinemic group and $n = 7$ in the basal insulin control group. There were no significant differences among hyperinsulinemic groups in any parameter when brain insulin action was blocked. During the hyperinsulinemic period insulin, NEFA, glucose infusion rate, EGP, net hepatic glucose balance, and net hepatic glycogenolytic flux differed ($P < 0.05$) in those groups from their basal period values and compared with the basal insulin group.

association with a brief increase in hepatic glycolysis and net hepatic lactate production (Supplementary Fig. 2). Blockade of hypothalamic insulin signaling (Fig. 4A) did not cause a detectable change in any parameter relevant to hepatic glucose metabolism in response to hyperinsulinemia (Fig. 3).

The eightfold rise in insulin markedly activated the insulin–brain–liver signaling axis (Fig. 4A) and the direct hepatic insulin–signaling cascade (Fig. 4B). In the liver of aCSF-treated animals, hyperinsulinemia caused the marked suppression of gluconeogenic enzyme mRNA and protein expression (Fig. 4C and D), a substantial induction of GK mRNA and protein expression (13- and 4-fold increases, respectively; Fig. 4E), suppression of GSK3 β mRNA and protein levels (30 and 25% reductions, respectively; Fig. 4F), and alterations in GSK3 β and GS phosphorylation (3.5-fold increase and 60% decrease, respectively; Fig. 4G). The ICV infusion of LY prevented the insulin-driven increase in

hepatic P-STAT3, indicating blockade of insulin action at the relevant neurons in the brain (Fig. 4A), but did not alter the direct activation of hepatic insulin signaling or the suppression of the gluconeogenic program (Fig. 4B–D). The blockade of hypothalamic insulin signaling partially blunted GK induction (resulting in only 8- and 2.5-fold increases in GK mRNA and protein expression; Fig. 4E) and completely prevented the suppression of GSK3 β mRNA and protein levels caused by eightfold insulin (Fig. 4F). The insulin-mediated alterations in phosphorylation of GSK3 β and GS were not affected by blockade of hypothalamic insulin action (Fig. 4F and G).

Stimulation of brain insulin signaling was evident quickly. Hypothalamic P-Akt was elevated to 2.3 ± 0.2 -fold basal after 60 min (vs. 2.7 ± 0.3 -fold after 240 min) of eightfold hyperinsulinemia. Hepatic P-STAT3 was unchanged (1.1 ± 0.3 -fold basal) after 1 h of elevated insulin but was increased to 2.6 ± 0.3 -fold by 4 h, consistent with

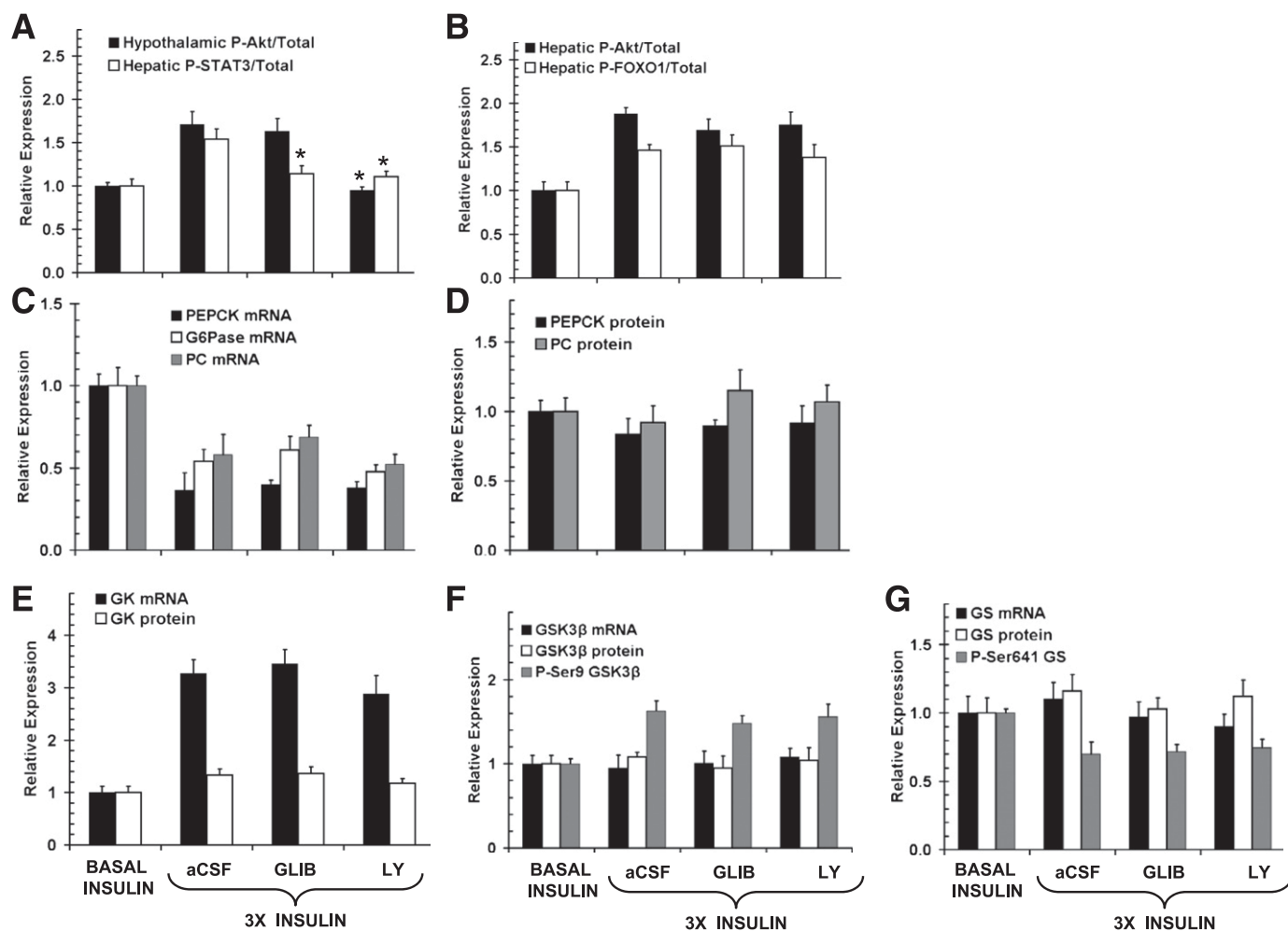


FIG. 2. Molecular response to a threefold increase in portal vein insulin infusion in animals characterized in Fig. 1. Hypothalamic insulin signaling indices (A) and hepatic insulin signaling indices (B). Hepatic gluconeogenic mRNA (C) and protein (D) expression. GK mRNA and protein expression (E); GSK3 β mRNA, protein, and phosphorylation levels (F) and GS mRNA, protein, and phosphorylation levels (G) are shown. Representative Western blots can be found in Supplemental Fig. 4. Values are means \pm SEM; *n* values are the same as in Fig. 1. **P* < 0.05 difference versus the aCSF-treated hyperinsulinemic group.

a lag in the activation of the STAT pathway (3,12). Likewise, ICV LY infusion blunted the rapid effect of brain insulin on hypothalamic P-Akt (1.3 ± 0.1 -fold basal). These data indicate that brain insulin action was increased for the full 4 h in the aCSF group and that it was blocked over the same time period in the LY group.

Thus, at higher insulin levels (eightfold basal), inhibition of gluconeogenic enzyme expression was regulated by the noncentral effects of insulin, GK induction was regulated by both central and peripheral insulin, and suppression of GSK3 β expression was controlled by brain insulin action, while the phosphorylation of GSK3 β and GS was not. Nevertheless, the direct effects of insulin on peripheral tissues (liver and fat) were entirely responsible for regulating glucose flux.

The effect of an eightfold increase in brain insulin action on the liver in the presence of a twofold increase in hepatic insulin action. The final experiments were designed to bring about a substantial elevation in brain insulin (eightfold) with only minimal hyperinsulinemia at the liver (twofold). In these experiments, portal vein and head artery insulin infusion increased insulin levels at the liver and brain to $\sim 32 \mu\text{U/mL}$ (Fig. 5A and B), whereas NEFA and glucagon were clamped at basal values (Fig.

5D and E). Arterial cortisol and catecholamine levels were basal and did not differ between groups (not shown). Similar glucose infusion rates were required to maintain euglycemia in both hyperinsulinemic groups ($\sim 6 \text{ mg/kg/min}$; Fig. 5F and G).

EGP was reduced by 50% and NHGB fell to zero by the end of the clamp in aCSF-treated hyperinsulinemic animals (Fig. 5H and I). The fall in EGP reflected a decrease in gluconeogenesis and glycogenolysis (Supplementary Fig. 3). The reduction in NHGB was a function of suppressed net hepatic glycogenolytic flux, as net hepatic gluconeogenic flux was not altered (Fig. 5J and K). Blockade of hypothalamic insulin signaling did not alter the metabolic response of the liver to insulin.

Insulin signaling in the brain was markedly increased (Fig. 6A), whereas direct liver insulin signaling was only moderately activated (Fig. 6B). In aCSF-treated animals, hyperinsulinemia suppressed gluconeogenic mRNA and protein levels (Fig. 6C and D), increased GK mRNA and protein expression (8- and 2.7-fold increases, respectively; Fig. 6E), decreased GSK3 β mRNA and protein levels (35 and 30% reductions, respectively; Fig. 6F), increased GSK3 β phosphorylation 2.4-fold (Fig. 6F), and reduced GS phosphorylation by 35% (Fig. 6G). Inhibition of

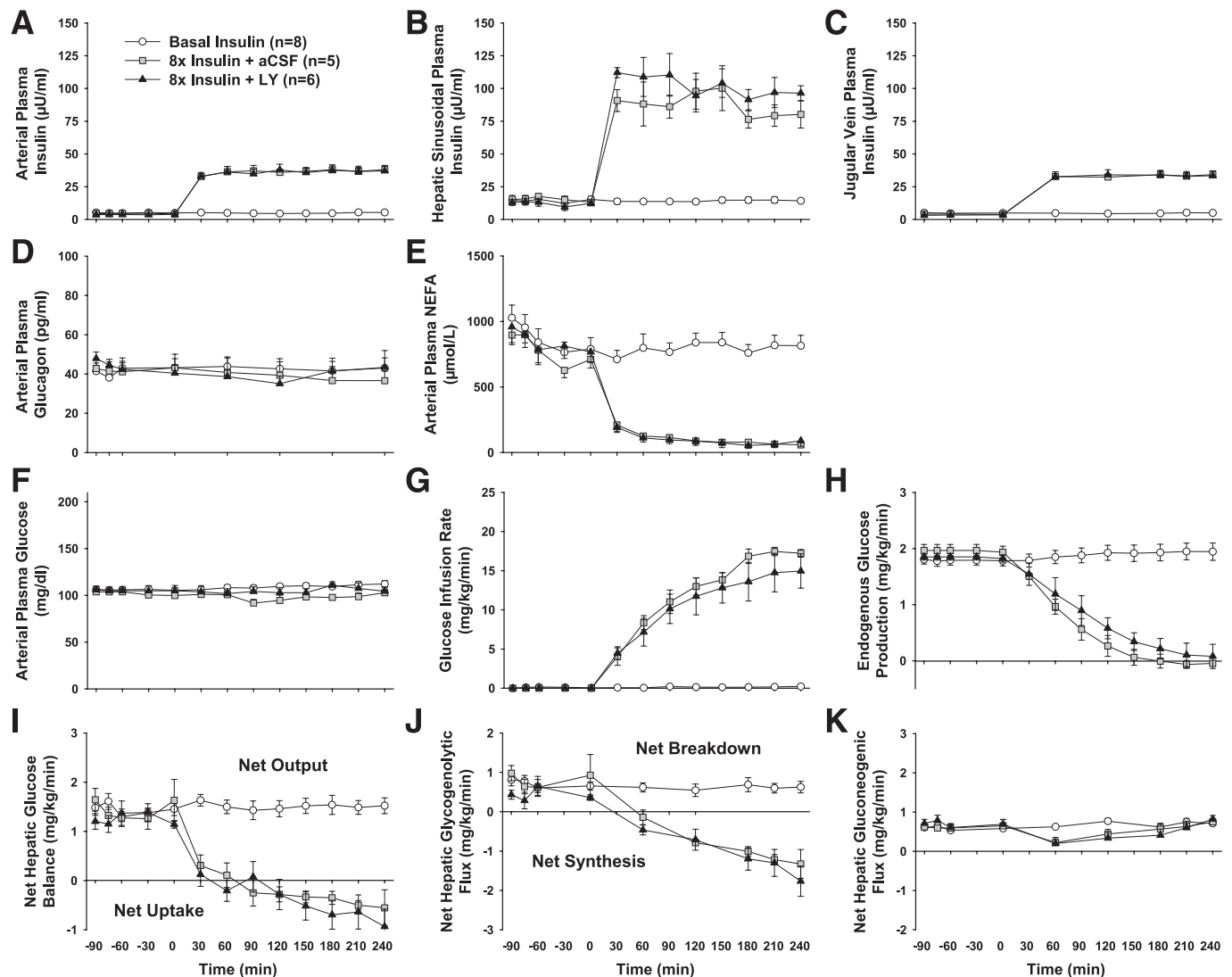


FIG. 3. Metabolic response to an eightfold increase in portal vein insulin infusion in dogs maintained on a pancreatic clamp (compared with basal insulin-infused controls). *A–E*: Circulating insulin, glucagon, and NEFA levels. *F–K*: Glucose flux parameters. Euglycemia was maintained (*F*) with similar rates of glucose infusion between groups (*G*). Eightfold insulin caused the complete suppression of EGP (*H*) and NHGB (*I*). The alteration in NHGB was primarily a function of sustained suppression of net hepatic glycogenolytic flux (*J*) with only a transient decrease in net hepatic gluconeogenic flux (*K*). Values are means \pm SEM; $n = 5, 6,$ and 8 in aCSF, LY, and BASAL INS groups, respectively. There were no significant differences among hyperinsulinemic groups in any parameter when brain insulin action was blocked. During the hyperinsulinemic period insulin, NEFA, glucose infusion rate, EGP, net hepatic glucose balance, and net hepatic glycogenolytic flux differed ($P < 0.05$) in those groups from their basal period values and compared with the basal insulin group.

hypothalamic insulin signaling blocked the insulin-driven increases in hypothalamic P-Akt and hepatic P-STAT3 (Fig. 6A), without altering direct hepatic insulin signaling or PEPCK/G6Pase mRNA/protein expression (Fig. 6B–D). Central inhibition of insulin action did, however, partially blunt the decrease in PC mRNA expression and the increase in GK mRNA and protein (resulting in only five- and twofold inductions, respectively; Fig. 6E). It also completely blocked the decreases in GSK3 β mRNA and protein (Fig. 6F) observed in the aCSF infusion group. In contrast, P-GSK3 β and P-GS levels were regulated by peripheral but not central insulin action (Fig. 6E and F).

These experiments confirm that both central and hepatic insulin action are involved in the hepatic gluco-regulatory gene expression response to acute (<4 h) physiologic hyperinsulinemia. However, once again, this interaction was not relevant to insulin's rapid inhibitory effect on

hepatic glucose metabolism, even in conditions designed to emphasize the role of central insulin signaling.

The molecular regulation of GK in response to hypothalamic and hepatic insulin action. Because GK induction was enhanced in response to simultaneous insulin sensing at both the liver and brain, relative to the liver alone, we examined key regulators of GK gene expression in animals from the second set of experiments (eightfold basal systemic hyperinsulinemia at all tissues). Physiologic hyperinsulinemia increased sterol regulatory element-binding protein 1c (SREBP1c) mRNA levels 2.4-fold, tended to increase SREBP1 protein levels (35%, not significant; Fig. 7A and B), increased peroxisome proliferator-activated receptor γ levels 3.5-fold (Fig. 7C), tended to increase both liver X receptor α mRNA expression and hepatocyte nuclear factor 4 phosphorylation (40%), and decreased small heterodimer partner (SHP)

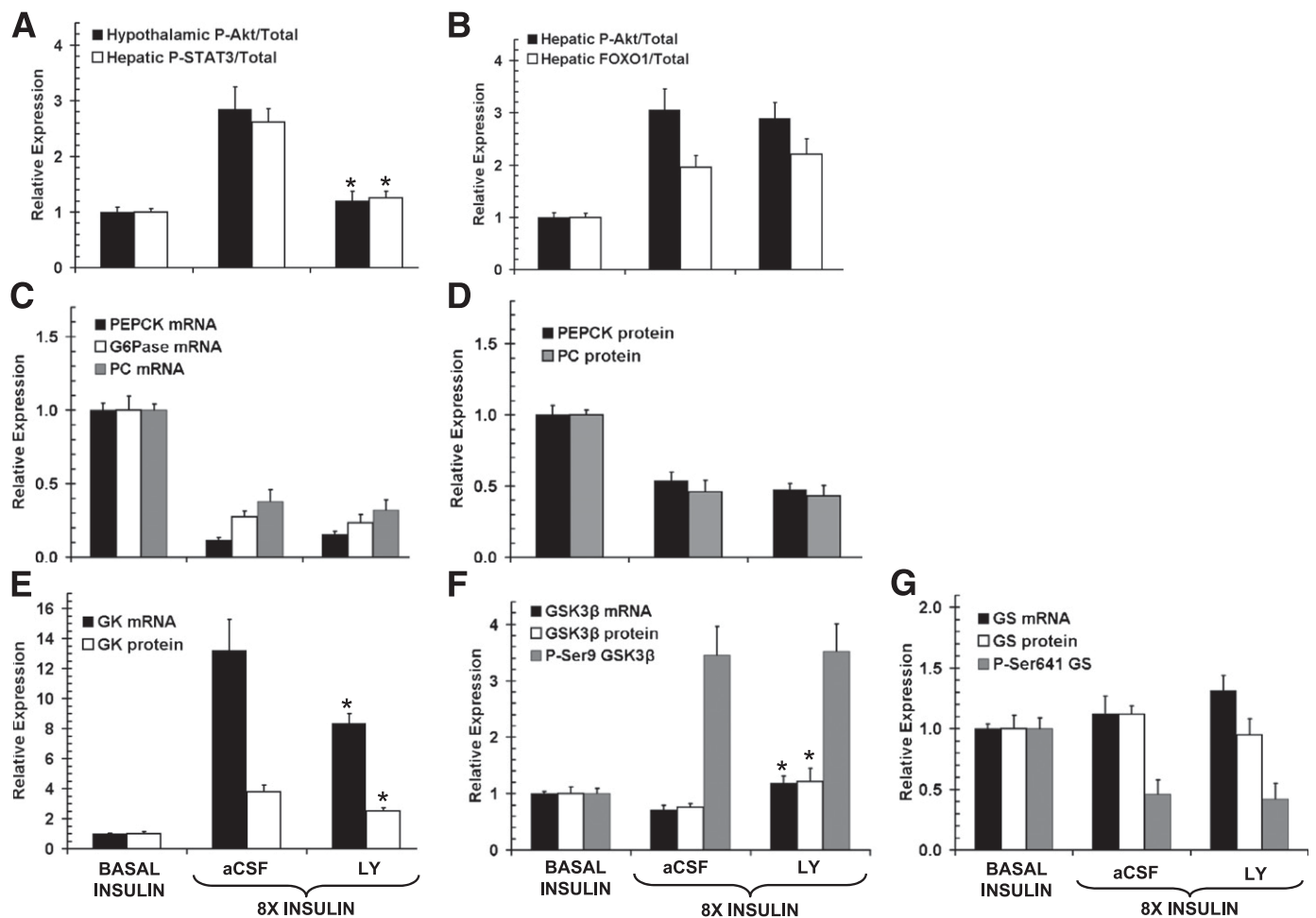


FIG. 4. Molecular response to eightfold increase in portal vein insulin infusion in animals characterized in Fig. 3. Hypothalamic insulin signaling indices (A) and hepatic insulin signaling indices (B). Hepatic gluconeogenic mRNA (C) and protein expression (D) are shown. Glucokinase mRNA and protein expression (E); GSK3 β mRNA, protein, and phosphorylation levels (F); and GS mRNA, protein, and phosphorylation levels (G) are shown. Representative Western blots can be found in Supplemental Fig. 5. Values are means \pm SEM; *n* values are the same as in Fig. 3. **P* < 0.05 difference versus the aCSF-treated hyperinsulinemic group.

protein expression by 40% (Fig. 7C–F). Blockade of hypothalamic insulin signaling prevented the decrease in SHP, a negative regulator of GK (21), without altering any of the other measured parameters (Fig. 7A–E).

DISCUSSION

Previously, we established that the brain of the dog can sense a physiologic rise in plasma insulin and communicate that information to the liver, thereby altering hepatic signaling and glycogen metabolism (12). However, previous dog (12) and rodent (1–11) studies characterized insulin's central inhibitory effects on EGP under circumstances that do not reflect the reality of physiologic hyperinsulinemia. Insulin levels are normally approximately threefold greater at the liver than the brain, but in the above-referenced clamp studies, insulin was infused peripherally, thus creating a state of relative hepatic insulin deficiency (similar or higher levels at the brain versus the liver). Therefore, our aim was to characterize, for the first time, the interaction between central and peripheral insulin action in the control of hepatic glucose metabolism during acute physiologic hyperinsulinemia when the liver/brain insulin ratio (3:1) was normal.

Inhibition of brain insulin action during modest (threefold) physiologic hyperinsulinemia did not detectably alter any measured parameter relevant to hepatic glucose metabolism. During more substantial (eightfold) hyperinsulinemia, however, the blockade of hypothalamic insulin signaling impacted the genetic regulation of hepatic GK and GSK3 β . Selective 10-fold brain hyperinsulinemia (brought about while hepatic insulin remained basal) previously (12) increased GK mRNA expression threefold. In the present studies, an 8-fold rise in insulin throughout the body increased GK mRNA 13-fold, but when hypothalamic insulin action was blocked, it only increased by 8-fold. Thus, the induction of hepatic GK mRNA during a physiologic rise in insulin appears to be attributable to the combined effects of central and peripheral insulin. Unlike GK, the acute suppression of GSK3 β mRNA was mediated exclusively by hypothalamic insulin action. These data suggest that the full response of both GK and GSK3 β expression to an acute increase in insulin requires hypothalamic insulin sensing. In contrast, increased phosphorylation of GSK3 β (leading to increased GS activity) and the suppression of gluconeogenic enzyme expression was completely driven by the peripheral rather than the central effects of insulin. Because selective brain

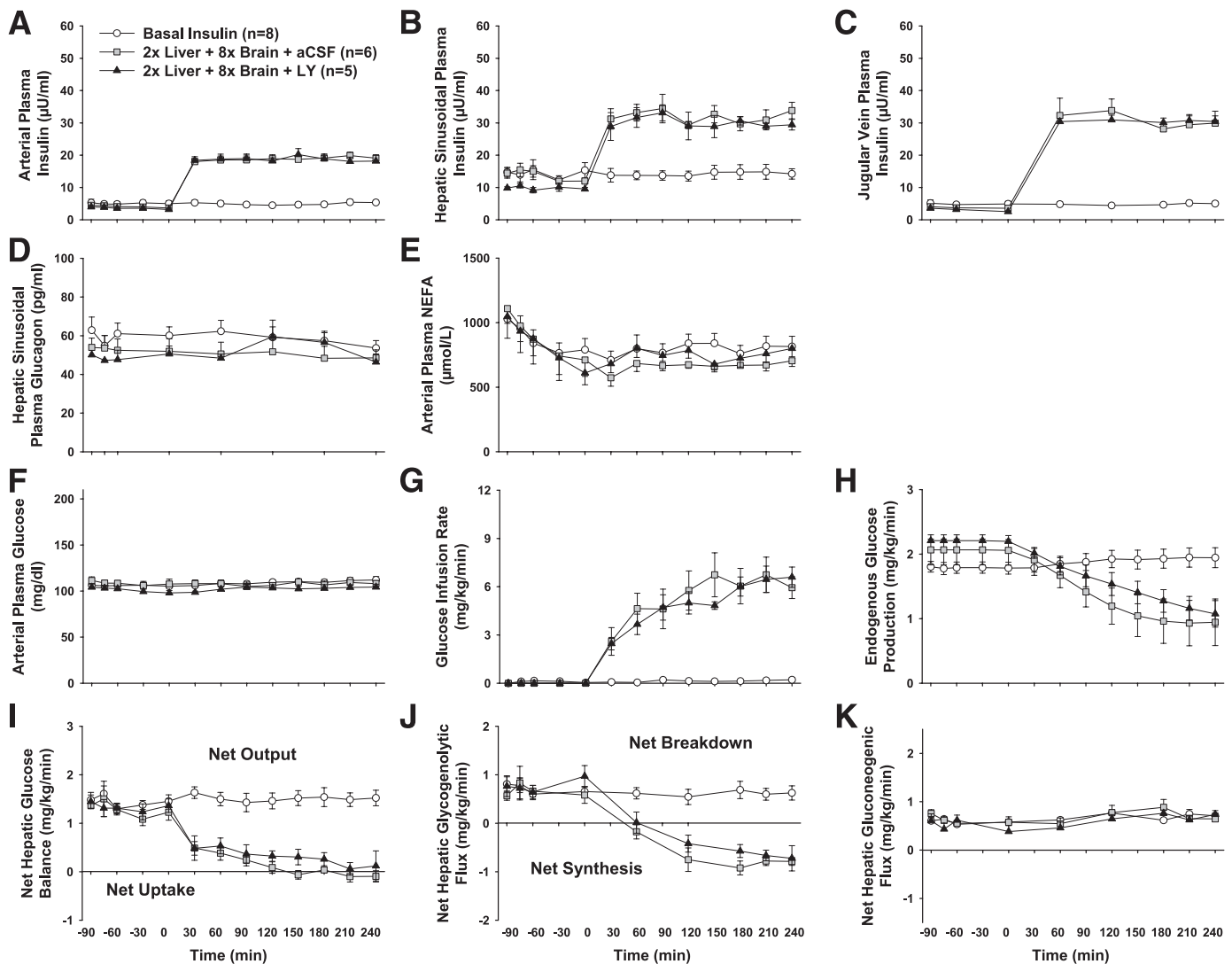


FIG. 5. Metabolic response to increased insulin infusion (portal vein plus head arteries) to bring about two times the hepatic-insulin and eightfold brain-insulin levels in dogs maintained on a pancreatic clamp (compared with basal insulin-infused controls). *A–E*: Circulating insulin, glucagon, and NEFA levels. *F–K*: Glucose flux parameters. Euglycemia was maintained (*F*) with similar rates of glucose infusion between groups (*G*). Insulin infusion suppressed both EGP (*H*) and NHGB (*I*). The alteration in NHGB was primarily a function of reduced net hepatic glycogenolytic flux (*J*) with no apparent effect on net hepatic gluconeogenic flux (*K*). Values are means \pm SEM; $n = 6, 5,$ and 8 in aCSF, LY, and BASAL INS groups, respectively. There were no significant differences between hyperinsulinemic groups in any parameter when brain-insulin action was blocked. During the hyperinsulinemic period, insulin, glucose infusion rate, EGP, net hepatic glucose balance, and net hepatic glycogenolytic flux differed ($P < 0.05$) in those groups from their basal period values and compared with the basal insulin group.

hyperinsulinemia can decrease hepatic GS phosphorylation and gluconeogenic enzyme expression (12), it would appear that the peripheral effects of insulin on liver and fat mask the effects of brain insulin action on the liver's glycogen synthetic and gluconeogenic programs.

Indicators of glucose flux (glucose infusion rate, EGP, net hepatic glucose balance, net hepatic glycogenolysis, and net hepatic gluconeogenesis) remained unchanged over time in control (basal insulin) experiments, but responded rapidly (by 30 min) and dose dependently to rises in insulin. This contrasts with the modest effect of a selective 10-fold rise in head insulin, which we previously (12) showed caused a decline in net hepatic glucose balance that was only apparent in the fourth hour of hyperinsulinemia. In the current study, systemic hyperinsulinemia increased brain insulin signaling within 60 min without affecting hepatic STAT3 phosphorylation. The latter fits with data (3,12) indicating that it takes several hours for brain-insulin effects on the

Janus kinase/STAT pathway to be manifest. Thus, it is clear that central nervous system-mediated changes in hepatic P-STAT3, and the downstream changes that it causes, cannot be responsible for the rapid effects of insulin on hepatic glucose metabolism. In agreement with earlier findings (15,22), a 50% fall in the level of gluconeogenic enzymes was not associated with reduced gluconeogenic (PEPCK) flux, demonstrating low control strength of the enzyme over gluconeogenesis. This finding fits with data from studies that showed that the gluconeogenic pathway was not suppressed by meal-associated hyperinsulinemia (23–25), despite insulin's ability to suppress PEPCK protein expression (15). Thus, the rapid effects of insulin on hepatic glucose metabolism in the current study were the consequence of glycogenolytic inhibition and the redirection of gluconeogenic carbon into glycogen, not a reduction of gluconeogenic flux to glucose-6-phosphate (15,20,26,27). Interestingly, although brain-insulin signaling created differences in GK and

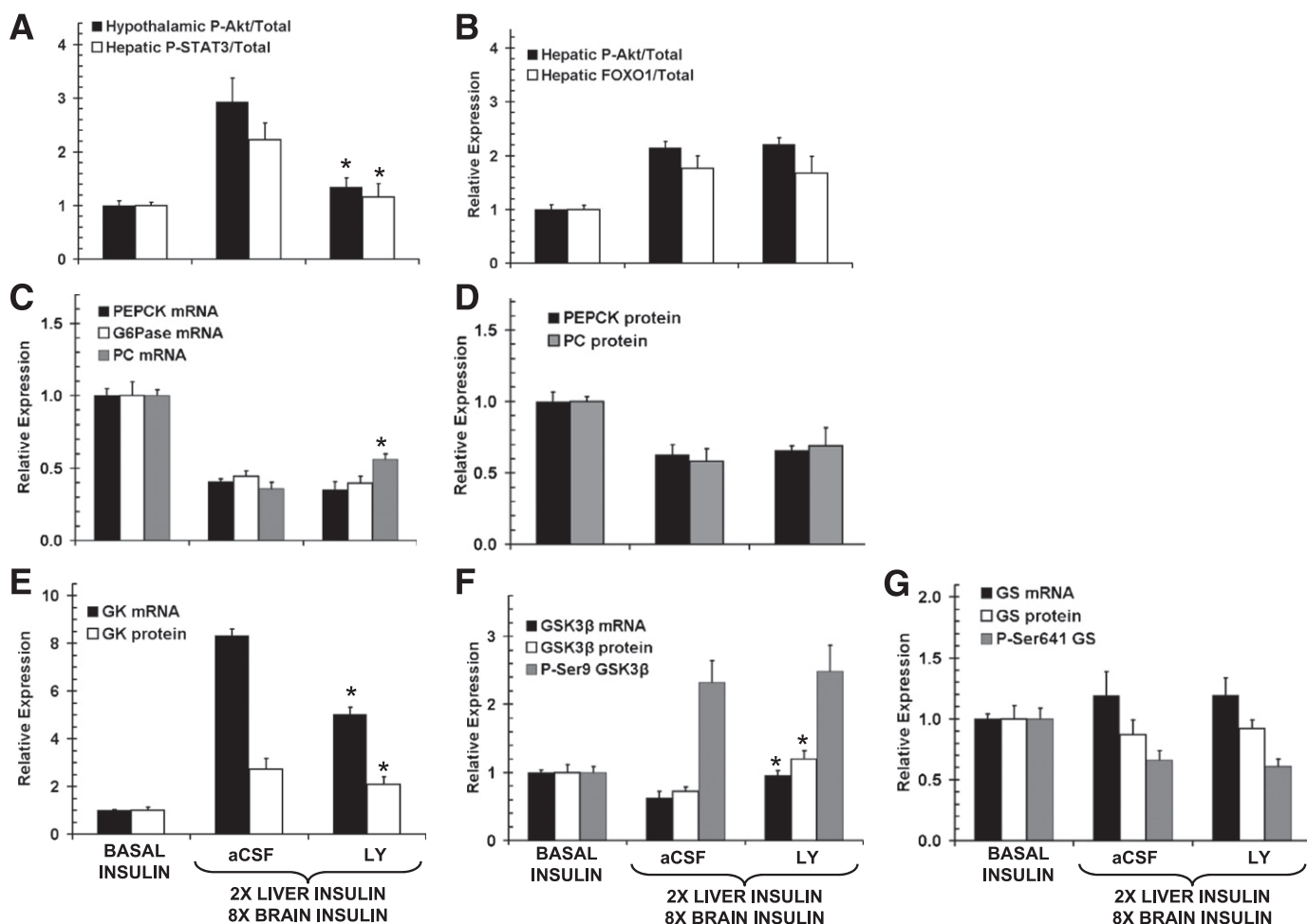


FIG. 6. Molecular response to hyperinsulinemia (two times liver, eightfold brain) for animals characterized in Fig. 5. Hypothalamic insulin signaling indices (A) and hepatic insulin signaling indices (B) are shown. Hepatic gluconeogenic mRNA (C) and protein (D) expression are shown. Glucokinase mRNA and protein expression (E); GSK3 β mRNA, protein, and phosphorylation levels (F); and GS mRNA, protein, and phosphorylation levels (G) are shown. Representative Western blots can be found in Supplemental Fig. 6. Values are means \pm SEM; *n* values are the same as in Fig. 5. **P* < 0.05 difference versus the aCSF-treated hyperinsulinemic group.

GSK3 β protein, those changes did not translate into an increase in hepatic glucose uptake, as was seen in the presence of selective brain hyperinsulinemia in our earlier study (12) or a decrease in EGP, as was shown in rodent studies (1–11). Because blockade of central insulin action had no significant inhibitory impact on any parameter relating to glucose flux, it would appear that it is the effects of insulin on fat and liver that drive the rapid changes in hepatic glucose flux seen in response to acute changes in insulin secretion in the normal animal.

There are several possible reasons why hypothalamic insulin action may have affected hepatic glucose metabolism in earlier (1–12) but not the present experiments. First, as noted, relative hepatic insulin deficiency (liver/brain insulin ratio of 1:1 in the rodent and 1:10 in the dog) was likely permissive for the hypothalamic-mediated effects of insulin on the liver. Clearly, under conditions of physiologic hyperinsulinemia (3:1 ratio), the peripheral effects of insulin (on liver and fat) mask the central effects of the hormone on hepatic glucose flux. A second point to consider is the duration of the impairment in hypothalamic insulin signaling. In the current study, in control (basal insulin) experiments, acute hypothalamic blockade (5-h duration) had no effect on hepatic signaling or glucose

metabolism, as was the case in the rat (8). More prolonged blockade, however, might have a greater impact. Thirdly, it should be kept in mind that we assessed the time course of insulin action on the liver, an important issue because the hormone's inhibitory effects on hepatic glucose metabolism begin within minutes. Rodent studies, in contrast, assessed insulin's effect on EGP under steady-state conditions after several hours of hyperinsulinemia. That being said, we saw no impact of brain-insulin action on EGP even in the fourth hour of hyperinsulinemia, whereas in the rat, there was an \sim 30% decrease at 2 h. Finally, glucose metabolism is different in rodents and large animals in several regards (e.g., the rodent has much higher basal rates of EGP, more complete glycogen depletion during fasting leading to an enhanced gluconeogenic contribution to EGP, differing hepatic neuroanatomy, etc.) (12,27,28), and it is possible that rodents have increased sensitivity of the gluconeogenic pathway to brain insulin-mediated inhibition compared with species with greater dependence on glycogenolysis.

Recently, oral administration of a K_{ATP} channel activator was shown to decrease EGP modestly (\sim 30%) in nondiabetic humans (inhibition was first apparent more than 5 h after oral diazoxide consumption during a

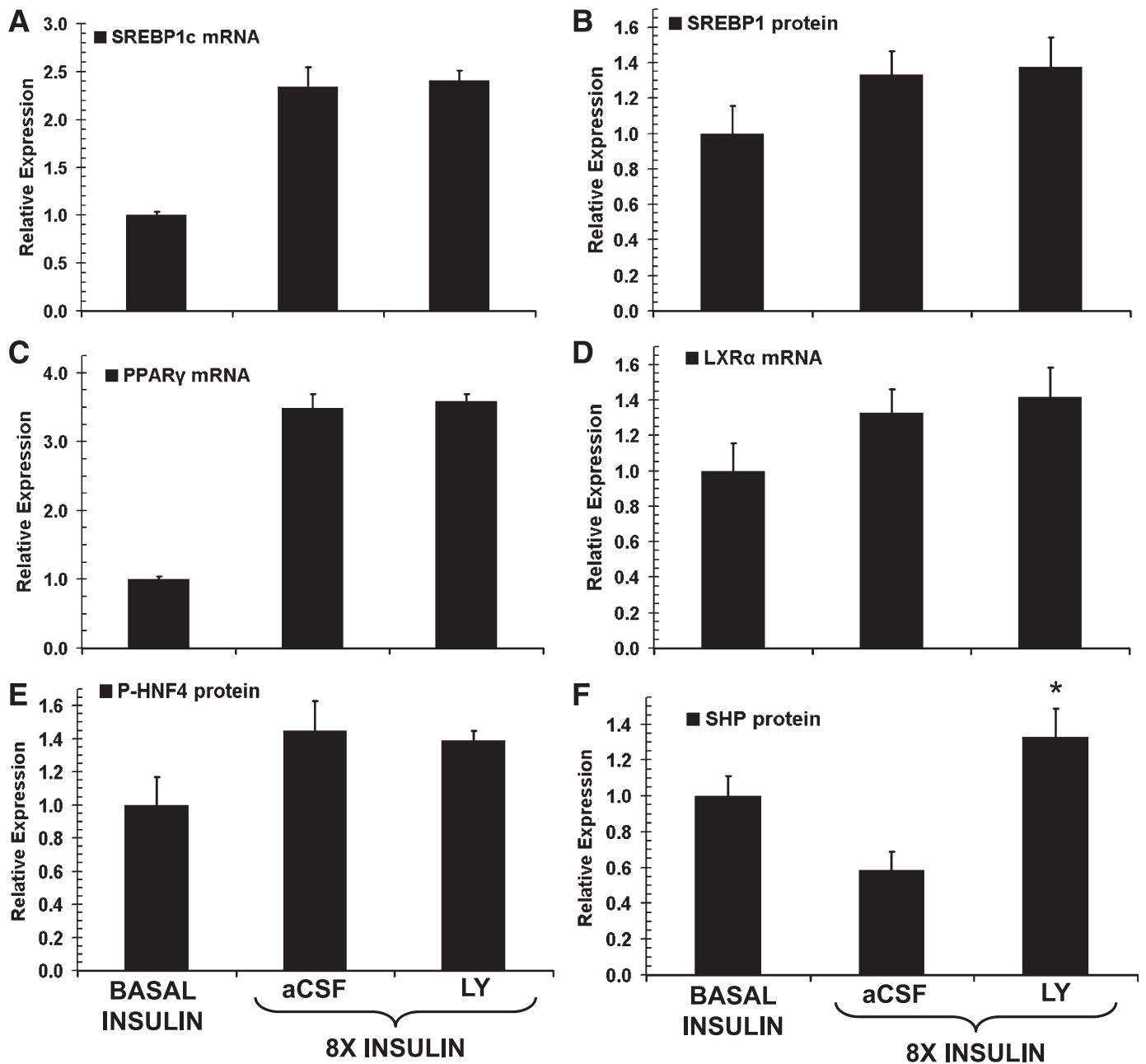


FIG. 7. Molecular regulators of GK expression in animals characterized in Fig. 3. SREBP1c mRNA (A) and SREBP1 protein (B) levels, peroxisome proliferator-activated receptor γ (PPAR γ) (C) and liver X receptor (LXR α) (D) mRNA expression, hepatocyte nuclear factor (HNF) 4 phosphorylation (E), and SHP protein expression (F) are shown. Values are means \pm SEM; *n* values are the same as in Fig. 3. **P* < 0.05 difference versus the aCSF-treated hyperinsulinemic group.

peripheral vein insulin clamp) (29). In rodent studies (8,11), hypothalamic K_{ATP} channel activation replicated the effect of ICV insulin administration on EGP, and co-administration of a K_{ATP} channel inhibitor blocked brain-insulin action. This suggests that brain insulin action has the potential to regulate liver glucose metabolism in the human, as in the rodent and the dog. It should be remembered, however, that the effect on EGP was slow to manifest, and the physiologic relevance of the finding is unclear, because the inhibition was observed during relative (1:1 ratio) hepatic insulin deficiency. Further, it is unknown whether the dose of diazoxide produced effects representative of physiologic or pharmacologic levels of insulin in the brain.

Although the current study addressed the acute ability of brain insulin action to affect the rapid response of the liver to hyperinsulinemia (such as might occur during a meal), it did not address the consequences of chronic changes in hypothalamic insulin action. Longer exposure of the canine brain to hyperinsulinemia may eventually bring about changes in hepatic glucose metabolism. In fact, the majority of studies investigating the effects brain insulin action on EGP have used models in which brain-insulin action was chronically altered (1–6,9,10). Long-term changes in the insulin–brain–liver signaling axis might alter the liver's ability to respond to acute changes in circulating insulin at the level of Akt phosphorylation, as has been suggested (10,30). It also appears that chronic hypothalamic

insulin resistance may contribute to the development of hepatic insulin resistance (2,4-6,9,10,30). On the other hand, the insulin-mediated regulation of hepatic glucose metabolism in response to fasting, insulin clamps, and a meal or glucose challenge is essentially normal in humans with liver transplants and dogs subjected to complete hepatic denervation (14,31-35), despite long-term absence of neural communication with the liver. Unfortunately, hepatic denervation is nonspecific, removing a variety of afferent and efferent nerve fibers, making conclusions from such studies difficult to draw.

Although this study demonstrates that acute physiologic hyperinsulinemia does not affect hepatic glucose metabolism in a euglycemic setting in the normal dog, brain-insulin action may be more important to the liver's response to insulin during times of nutrient excess (hyperglycemia), when insulin is normally secreted. Although the regulation of hepatic glucose metabolism during the acute meal response begins too quickly (within minutes) to involve genetic mechanisms, central insulin's effects on genes relevant to glucose uptake (GK) and glycogen synthesis (GSK3 β) may become important to the hepatic response to subsequent meals. In addition, nongenomic effects of brain-insulin action may become apparent under hyperglycemic conditions, as they did under hypoglycemic conditions when brain-insulin action was shown to augment the rapid sympathetic counterregulatory response to low blood glucose (36-38).

In summary, we demonstrate for the first time that genetic regulation of GSK3 is mediated by hypothalamic insulin action, whereas the induction of GK expression during physiological hyperinsulinemia (normal liver/brain insulin ratio) is the result of insulin's effects at both the hypothalamus and the liver. Despite these genetic effects, the regulation of hepatic glucose metabolism in response to an acute physiologic rise in insulin was not impaired by the acute inhibition of hypothalamic insulin action. This suggests that acute activation of hypothalamic insulin signaling is not required for insulin's rapid inhibitory effect on EGP in the normal dog. Our studies do not rule out an important role for brain insulin action in other circumstances. Studies are needed to determine the importance of chronic changes in central insulin signaling to the acute control of liver glucose metabolism under physiologic conditions. Likewise, the role of central insulin signaling in the response of the liver to meal (glucose) ingestion also needs to be clarified.

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