

Evidence against a Physiologic Role for Acute Changes in CNS Insulin Action in the Rapid Regulation of Hepatic Glucose Production

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This Perspective will discuss the physiologic relevance of data that suggest CNS insulin action is required for the rapid suppression of hepatic glucose production. It will also review data from experiments on the conscious dog, which show that although the canine brain can sense insulin and, thereby, regulate hepatic gluco-regulatory enzyme expression, CNS insulin action is not essential for the rapid suppression of glucose production caused by the hormone. Insulin's direct hepatic effects are dominant, thus it appears that insulin's central effects are redundant in the acute regulation of hepatic glucose metabolism.

Introduction

Insulin is a primary regulator of hepatic glucose metabolism in healthy individuals. Failure of the liver to appropriately respond to insulin (hepatic insulin resistance) is an underlying cause of the increased hepatic glucose production (HGP) and hyperglycemia associated with type 2 diabetes. Therefore, elucidating mechanisms that are fundamental to the insulin-mediated control of HGP and understanding the loss of this control in the insulin resistant state remain priorities in the field. Insulin is known to rapidly suppress HGP through its direct action at the liver and also through indirect effects thought to be mediated primarily at adipose tissue and the α -cell (Figure 1) (Bergman, 1997; Cherrington, 1999; Rizza, 2010). In the past decade, studies in rodents have suggested that insulin signaling in the central nervous system (CNS) also has the ability to modify neural input to the liver and, as a result, suppress HGP (Guo et al., 2009; Hill et al., 2010; Inoue et al., 2006; Koch et al., 2008; Könnner et al., 2007; Lin et al., 2010; Obici et al., 2002a, 2002b; Ono et al., 2008; Park et al., 2009; Pocai et al., 2005). In addition, CNS insulin action has been suggested to suppress adipose tissue lipolysis (Scherer et al., 2011) and α -cell glucagon secretion (Paranjape et al., 2010), thereby providing additional potential mechanisms for central control of HGP (Figure 1). Furthermore, some studies of CNS insulin action have suggested that the central effect of insulin is required for the rapid suppression of HGP in response to increased insulin secretion (Obici et al., 2002b; Pocai et al., 2005). Moreover, it has been suggested that targeting CNS insulin resistance may be of value in the treatment of human type 2 diabetes (Sandoval et al., 2009).

Whether insulin's central arm is conserved in, or relevant to, the regulation of hepatic glucose production in humans is unknown. To help address this uncertainty, we have been studying CNS insulin action and its effects on HGP in the conscious dog, a model in which glucose metabolism is similar to the human in several regards (e.g., the basal rate of HGP and slow depletion of liver glycogen). Our studies suggest that the insulin-CNS-liver signaling axis characterized in the rodent is conserved in the dog, but that the rapid and marked inhibitory

effect of insulin on HGP does not depend on this mechanism for its effect. This Perspective will first discuss the data, which have been interpreted to support the notion that insulin's central action is necessary for the rapid regulation of HGP. We will then focus on limitations of the methods that have been used to study brain insulin action on the liver *in vivo*. Finally, we will review the evidence from experiments in humans and dogs indicating that acute changes in CNS insulin action are not essential for the hormone's rapid inhibitory control of hepatic glucose production; instead this mechanism appears to be masked in the presence of insulin's prominent non-CNS effects.

The Case for CNS Insulin Action in the Acute Regulation of HGP

Although classic studies established that CNS insulin can regulate energy homeostasis (Woods et al., 1979) and pancreatic hormone secretion (Woods and Porte, 1975), until recently there had been little evidence indicating that CNS insulin could directly regulate HGP. Interest in insulin's central action was significantly enhanced by a provocative study in which rats were infused with either vehicle or insulin through an intracerebroventricular (ICV) cannula (Obici et al., 2002b). By the fourth hour of treatment, ICV insulin had not caused any alteration in glucose metabolism or pancreatic hormone secretion when compared to vehicle-infused controls. Both groups were then subjected to a pancreatic clamp, in which somatostatin was used to inhibit endogenous insulin and glucagon secretion, and insulin, but not glucagon, was replaced via a peripheral vessel at a rate (1.0 mU/kg/min) calculated to maintain arterial plasma insulin levels at basal values. During the last hour of the 2 hr clamp (the sixth hour of infusion), ICV insulin was associated with a 30% decrease in HGP relative to the rate in an ICV vehicle-infused control group (which decreased from basal by ~10%). In addition, in a separate experiment of the same duration, interruption of central insulin action (by ICV infusion of hypothalamic insulin signaling or K_{ATP} channel inhibitors for six hours) substantially reduced suppression of HGP caused by hyperinsulinemia resulting from peripheral vein infusion of

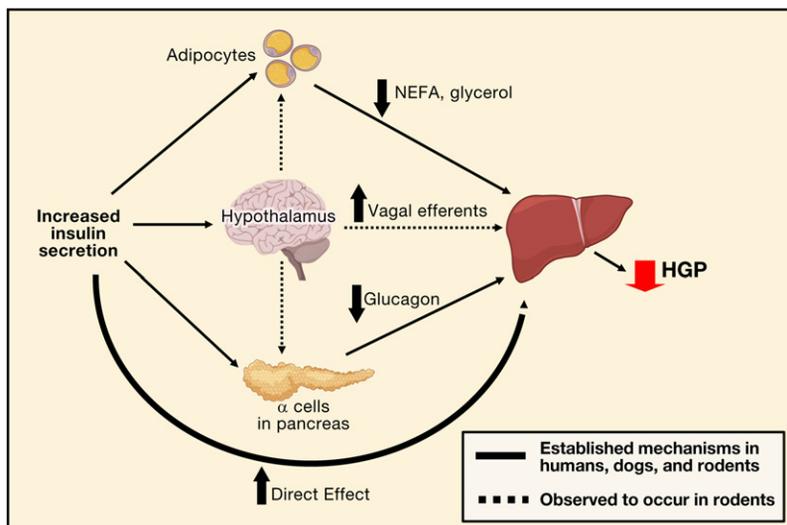


Figure 1. The Control of Hepatic Glucose Production by Insulin

Insulin suppresses HGP by both direct (hepatic insulin receptor signaling) and indirect effects. The indirect effects of insulin on the liver include inhibition of lipolysis in adipocytes, reduction in glucagon secretion by the α -cells of the pancreas, and (in rodents) a decrease in vagal efferent signaling to the liver per se. In addition, insulin action in the brain may also inhibit glucagon secretion and decrease lipolysis, thus reinforcing insulin's direct actions on these tissues. Not shown are the effects of insulin on gluconeogenic substrate supply from muscle, which are typically minor unless insulin is increased dramatically (Edgerton et al., 2009a). The relative contribution of each pathway to the control of liver glucose metabolism may vary depending on experimental conditions, metabolic state, and differences in species.

insulin at 3.0 mU/kg/min for the last 2 hr of the study (Obici et al., 2002b). These data led the authors to conclude that activation of the insulin-brain-liver signaling axis is required for the rapid action of insulin on glucose production.

Genetic manipulation of the insulin receptor (IR) or insulin-mediated events in the entire CNS (Brüning et al., 2000; Koch et al., 2008; Lin et al., 2011), the hypothalamus (Obici et al., 2002a; Ono et al., 2008), or specific populations of hypothalamic neurons (Hill et al., 2009, 2010; Könnner et al., 2007; Lin et al., 2010) have supported the notion that CNS insulin action plays a role in the suppression of HGP. This suppression requires intact vagus efferent nerves in both rats and mice (Pocai et al., 2005) but may be independent of hepatic muscarinic acetylcholine (the major neurotransmitter released from vagal efferent nerve endings) receptor signaling (Li et al., 2009). In any case, it has been suggested that the brain insulin-mediated reduction in HGP requires hepatic IL6 expression (Inoue et al., 2006; Könnner et al., 2007), hepatic STAT3 phosphorylation (Inoue et al., 2006; Koch et al., 2008) and appears to be achieved via suppression of gluconeogenesis (Pocai et al., 2005). The latter was associated with reductions in the expression of gluconeogenic mRNA (PEPCK, G6Pase) (Inoue et al., 2006; Pocai et al., 2005) and protein (G6Pase) (Könnner et al., 2007). In addition, rodent studies have indicated that long-term decreases in CNS insulin action can impair the response of HGP to insulin in models of insulin resistance (Hill et al., 2009, 2010; Koch et al., 2008; Könnner et al., 2007; Lin et al., 2010; Ono et al., 2008; Park et al., 2009). Furthermore, chronic increases in CNS insulin action by either genetic overexpression of hypothalamic insulin signaling proteins (Gelling et al., 2006) or prolonged ICV insulin infusion (Park et al., 2009) improved the acute response to insulin-regulated glucose metabolism in diabetic animals.

Methodological Limits to In Vivo CNS Insulin Studies

Insulin and glucagon distribution during the peripheral insulin clamp. The peripheral basal insulin clamp frequently used in rodents and humans involves the infusion of somatostatin and insulin into a peripheral blood vessel. Unfortunately, this ablates

the normal physiologic gradient of insulin and glucagon that exists between hepatic and non-hepatic tissues. Under normal circumstances, insulin and glucagon are both secreted into the portal vein and are subject to considerable

extraction by the liver. For insulin, this creates a gradient across the liver such that hepatic insulin levels are \sim 3-fold that of the insulin levels at nonhepatic tissues, including the brain (Horwitz et al., 1975; Moore et al., 2002). During a basal insulin clamp protocol (peripheral vein insulin infusion at a rate of 1.0 mU/kg/min), arterial insulin levels are maintained at basal values, but because endogenous insulin secretion is eliminated, marked hepatic insulin deficiency occurs simultaneously (Figure 2). The hyperglycemia expected to result from hepatic insulin deficiency can be avoided if glucagon, the primary driver for basal HGP in rodents, dogs, and humans (Brand et al., 1995; Cherrington et al., 1978; Liljenquist et al., 1977) is not replaced. Thus, in studies in which this approach was used, the central effects of ICV insulin were observed at a time when the liver was deficient in two of its primary regulatory signals, insulin and glucagon.

CNS insulin action has also been studied using the hyperinsulinemic clamp, with or without somatostatin infusion. In this setting, insulin is infused into a peripheral blood vessel at a rate (typically 3.0–3.6 mU/kg/min) designed to cause arterial hyperinsulinemia. The physiologic insulin gradient is again disrupted because the fold increase in insulin at the liver will be markedly less than that at the brain, a situation that does not occur when insulin secretion increases. This relative hepatic insulin deficiency is likely to lead to an overestimation of the importance of insulin's nonhepatic (including CNS) mechanisms in regulating HGP relative to what occurs in normal physiology. In addition, hyperinsulinemia resulting from insulin infusion has been shown to decrease glucagon secretion (Cherrington, 2001), perhaps due, in part, to CNS insulin-mediated effects on the α -cell (Paranjape et al., 2010). Thus, a fall in hepatic glucagon levels would also be expected to occur during a hyperinsulinemic clamp, regardless of whether or not somatostatin is infused, and this may contribute, directly or indirectly, to the suppression of HGP. Given these considerations, it is difficult to judge, based on existing clamp data in the rodent, the relevance of CNS insulin signaling to the control of HGP under normal physiologic conditions (i.e., when relative levels of insulin and glucagon at the liver are normal).

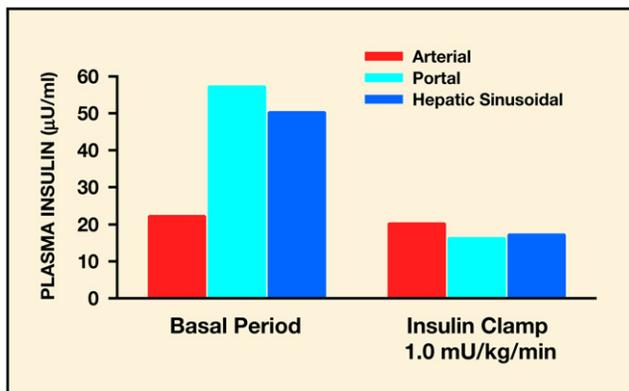


Figure 2. Hepatic and Nonhepatic Insulin Levels during the Basal State and during the Basal Peripheral Vein Insulin Clamp

Arterial data are redrawn from Obici et al. (Obici et al., 2002b), and hepatic portal vein and hepatic sinusoidal levels were estimated based on the arterial-portal insulin gradients resulting from endogenous secretion or peripheral vein infusion (Chu et al., 2004; Moore et al., 2002). Because of high first-pass insulin clearance, hepatic insulin levels are ~3-fold greater than concentrations in the artery. During the clamp, somatostatin is infused and nonhepatic (arterial) insulin levels are maintained at basal, while liver insulin levels are markedly deficient. Stimulation of hepatic glucose production can be avoided if glucagon is not replaced.

ICV and intrahypothalamic (IH) insulin infusions. ICV/IH infusions are experimental tools that have been used extensively to study the effects of a selective rise in insulin in the CNS on peripheral (i.e., non-CNS) glucose metabolism. Insulin infused directly into the brain can reach hypothalamic centers relevant to whole-body glucose metabolism, yet this manner of delivery is nonphysiologic in terms of route and, probably, dose. It is unknown how insulin perfusion of CNS tissue during ICV/IH administration compares to the perfusion present when the hormone enters physiologically through the vasculature. ICV/IH infusions may well cause nonphysiologic regionalization of the administered insulin. This is an important consideration because although hypothalamic tissue is easily accessed during ICV/IH infusion, access to distal centers in the brain that can regulate glucose metabolism may be limited (Levin and Sherwin, 2011). This limitation can be avoided through the infusion of insulin into the carotid and vertebral arteries, allowing brain insulin levels to be selectively elevated using the physiological route.

Blockade of brain insulin signaling. There are several considerations inherent in ICV administration of PI3K inhibitors (e.g., LY294002) to disrupt CNS insulin action. First, insulin can activate signaling via pathways other than PI3K. This may not be a significant issue, however, because Obici et al. found that PI3K but not MAP kinase was involved in the insulin-brain-liver signaling axis (Obici et al., 2002b). Second, the neurons targeted by insulin reaching the brain from the circulation may differ from those targeted by a PI3K inhibitor given ICV, because ICV delivery results in concentrations at ventricular surfaces that are likely to be greater than those occurring at deeper sites. In previous studies, however, administration of inhibitors generated similar effects regardless of site of infusion (ICV or IH), suggesting that the neurons in the hypothalamus that are responsible for the effects of brain insulin at the liver are accessible to small-molecule inhibitors via the ICV route, and that possible blockade of PI3K at nonhypothalamic sites did not

contribute to the observed brain insulin response (Pocai et al., 2005). Third, PI3K is not only involved in insulin signaling (e.g., leptin also signals through this pathway) (Hawkins et al., 2006; Warne et al., 2011), but this is probably not a problem when one is examining the effects of a selective rise in insulin if a baseline LY294002 control experiment is included. Finally, LY294002 could have off-target (non-PI3K) effects (Gharbi et al., 2007). Studies performed by the Rossetti group (Obici et al., 2002b; Pocai et al., 2005), however, showed that the effects of brain insulin signaling on HGP could be blocked as effectively by ICV infusion of PI3K inhibitors as by ICV or IH injection of insulin antibodies, insulin receptor antisense oligonucleotides, or K_{ATP} channel inhibitors. This suggests that ICV administration of PI3K inhibitors to acutely block brain insulin action is a reasonable approach.

Evidence Against a Role for CNS Insulin Action in the Acute Control of HGP in Large Animals

In light of the array of rodent data referred to previously, there is a need to critically evaluate the role of CNS insulin action in the regulation of HGP in humans. Obviously, such experiments are extremely difficult to carry out. Therefore, in order to address this question in a large animal model, we have been examining central insulin action in the dog. This model allows for the infusion of insulin into the hepatic portal vein and the carotid and vertebral arteries and for the calculation of net hepatic glucose balance (i.e., the net of hepatic glucose production and uptake) to complement tracer-derived estimation of HGP. To date, we have evaluated the importance of CNS insulin action in a variety of ways using the portal vein pancreatic clamp technique under euglycemic conditions. Since glucagon was clamped at a basal value, this would cause us to underestimate the importance of CNS insulin action on HGP to the extent that a rise in hypothalamic insulin suppresses glucagon secretion (Paranjape et al., 2010). Nevertheless, use of the portal vein pancreatic clamp in combination with a glucose clamp allows us to carefully assess the regulatory importance of the insulin-brain-liver signaling axis (Figure 1), independent of changes in circulating glucagon or glucose levels.

In studies performed a number of years ago, we first examined the mechanism by which a selective rise in insulin at nonhepatic tissues (i.e., brain, muscle, and fat) can suppress HGP in the presence of basal insulin at the liver (Sindelar et al., 1997). Initially, in the control period, insulin and glucagon were infused at basal rates into the portal vein of dogs. During the experimental period, the portal vein insulin infusion rate was decreased, and at the same time insulin was infused into a peripheral vein to bring about a selective ~3-fold increase in insulin at nonhepatic tissues while maintaining basal insulin levels at the liver. Selective nonhepatic hyperinsulinemia suppressed net hepatic glucose output and HGP with a time course corresponding to the time course of the decrease in circulating NEFA levels. When this fall in NEFA was prevented using intralipid infusion, the suppression of net hepatic glucose output and HGP was blocked. These data and those of others (Rebrin et al., 1996) suggest that the insulin-driven suppression of lipolysis (and not CNS insulin-driven input to the liver) was responsible for the acute inhibition of hepatic glucose production in response to modest nonhepatic hyperinsulinemia.

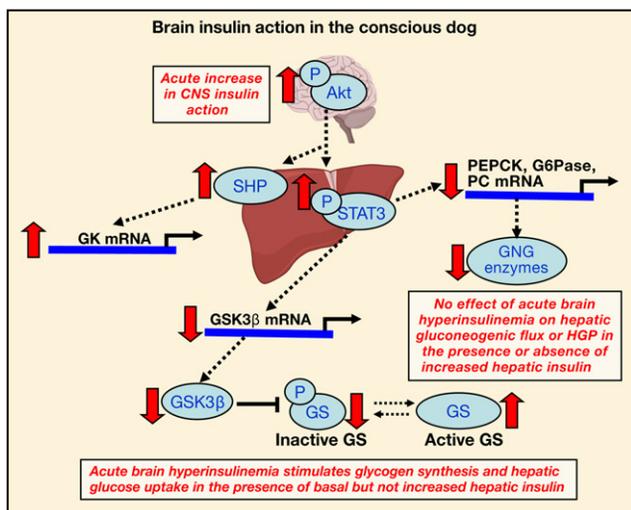


Figure 3. Model for the Insulin-Brain-Liver Signaling Axis in the Dog Increased hypothalamic insulin signaling causes an increase in hepatic signal transducer and activator of transcription 3 (STAT3) phosphorylation. Phosphorylated STAT3 reduces the gluconeogenic gene and protein expression of phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase), and pyruvate carboxylase (PC). Glycogen synthase kinase-3 beta (GSK3 β) gene and protein expression are also reduced by STAT3, leading to the activation of glycogen synthase (GS). In addition, brain insulin induces glucokinase (GK) gene expression; this effect is associated with a decrease in SHP protein, a negative regulator of GK expression. During a basal pancreatic clamp (hepatic sinusoidal insulin and glucagon and arterial NEFA and glucose, all clamped at basal values), brain insulin action does not acutely alter gluconeogenesis or hepatic glucose production in the dog, despite suppressing gluconeogenic gene expression. Central insulin action does, however, stimulate hepatic glucose uptake and glycogen synthesis associated with the genetic regulation of GSK3 β . During physiologic hyperinsulinemia, brain insulin action does not impact the rapid suppression of hepatic glucose metabolism.

In another study, we assessed the relative importance of the direct (hepatic) and indirect (nonhepatic; i.e., muscle, adipose, and CNS) effects of insulin on HGP (Edgerton et al., 2006). Dogs were first maintained on a basal pancreatic clamp using portal vein infusion of insulin and glucagon. During the experimental period, the insulin infusion was switched from the portal vein to a leg vein, thereby bringing about an \sim 2-fold increase in arterial insulin (i.e., at nonhepatic tissues) and an \sim 50% decline in insulin at the liver. Net hepatic glucose output and HGP were markedly increased as a result of hepatic insulin deficiency, rather than suppressed as a result of increased nonhepatic (including the CNS) hyperinsulinemia. In another group of animals, the basal insulin infusion was switched from the portal vein to the carotid and vertebral arteries, rather than a leg vein, thereby increasing CNS insulin 4-fold. Once again, there was a 50% fall in insulin at the liver, and net hepatic glucose output and HGP increased as they did when the insulin infusion was switched to a leg vein. Thus, additional CNS insulin enrichment had no effect on the metabolic response to hepatic insulin deficiency. These data clearly indicate the importance of the direct effect of insulin on the liver to the basal rate of glucose production.

Given the design of the above studies, it is possible that we did not detect CNS insulin-mediated regulation of hepatic glucose metabolism because the brain hyperinsulinemia that we em-

ployed was too low. Thus, we set about to examine the effects of greater brain hyperinsulinemia on the liver. Our first aim was to determine if the insulin-brain-liver signaling axis characterized in rodents exists in the dog. To do so, we infused insulin via an ICV cannula at the same dose observed to suppress HGP in the rat (Obici et al., 2002b) during a basal intraportal insulin and glucagon clamp. ICV insulin was able to increase hypothalamic pAkt 3.5-fold and hepatic pSTAT3 2.9-fold and suppress the mRNA expression of pyruvate carboxylase, G6Pase, and PEPCK by \sim 30%–60% (Ramnanan et al., 2011a). Even though CSF insulin levels increased \sim 200 fold, far above what would ever happen in the intact animal, and despite the fact that gluconeogenic mRNA expression was suppressed (as in rodents), ICV insulin treatment did not significantly alter either net or absolute basal glucose production over the 4h observation period. Of importance, however, these studies proved that the canine brain can sense acute increases in plasma insulin and, as a result, modify glucoregulatory gene expression in the liver.

As noted earlier, there are inherent difficulties in interpreting the physiologic relevance of ICV insulin infusion. Therefore, our next goal was to evaluate whether a substantial, but more physiologic (in terms of vascular route and dose), increase in CNS insulin could modify hepatic glucose flux in the dog. Therefore, we infused insulin bilaterally into the carotid and vertebral arteries to increase the CNS insulin level 10-fold, while at the same time using clamp techniques to fix hepatic sinusoidal insulin and glucagon, and arterial glucose and NEFA levels, at basal values (Ramnanan et al., 2011a). The experiment was carried out both in the presence (ICV aCSF infusion) and absence (ICV infusion of a PI3K inhibitor) of hypothalamic insulin signaling. Brain hyperinsulinemia did not alter hepatic glucose production, gluconeogenesis, or glycogenolysis but was able (in the fourth hour) to reduce net hepatic glucose balance as a result of altered net hepatic glycogen metabolism. That CNS insulin reduced net hepatic glucose balance without altering HGP implied that the effect was due to the stimulation of hepatic glucose uptake and glycogen synthesis. The slow time course of the effect suggested regulation at the genetic level (Figure 3). In accord with that finding, we observed that brain hyperinsulinemia increased glucokinase mRNA and decreased the mRNA and protein levels of GSK3 β , another STAT3-regulated gene (Moh et al., 2008). Reduced GSK3 β protein was associated with decreased glycogen synthase phosphorylation, which resulted in enhanced glycogen synthase activity (Figure 3). ICV administration of a PI3K inhibitor (LY294002) had no effect in the presence of basal insulin levels but inhibited hypothalamic Akt phosphorylation and blocked the centrally mediated downstream molecular consequences of increased insulin in the liver, as well as the associated changes in glucose kinetics.

The dog and rodent studies described above all defined brain insulin's effects in the context of relative hepatic insulin deficiency (Figure 2). It should be kept in mind that when the pancreas secretes insulin into the portal vein, the absolute magnitude of the rise in plasma insulin at the liver will be approximately three times greater than at the brain. It is, therefore, possible that when appropriate hyperinsulinemia occurs at the liver, the effects of brain hyperinsulinemia will be masked, meaning that CNS insulin action would have no impact on the

control of HGP in the presence of direct hepatic insulin action. Alternatively, there may be interaction between the effects of acute changes in brain and liver hyperinsulinemia, such that both are required to bring about a full hepatic response.

In light of these uncertainties, we determined the role of brain insulin action in the regulation of HGP in the context of a simulated increase in insulin secretion, such that the level of the hormone in plasma increased simultaneously by the same fold at all tissues of the body, including brain, muscle, fat, and liver (Cherrington, A.D., unpublished data; Edgerton et al., 2009b; Ramnanan et al., 2011b). When both brain hyperinsulinemia and hepatic hyperinsulinemia were present, direct hepatic insulin signaling controlled the hepatic gluconeogenic program, brain insulin signaling controlled hepatic GSK3 β expression, and the induction of glucokinase expression was under dual brain- and hepatic insulin-mediated regulation. Nongenomic effects of brain insulin action on hepatic glucose production were not apparent, however, and CNS insulin action had no bearing on the acute suppression of lipolysis during physiologic hyperinsulinemia. Because of the lack of effects of brain insulin action on the liver, adipose tissue, or muscle, the rapid regulation of hepatic glucose metabolism by physiologic increases in insulin occurred independent of the acute increase in CNS insulin action, despite differential genetic regulation. Our data thus indicate that the acute effects of CNS insulin action on whole-body glucose kinetics are overwhelmed by the consequences of the hormone's noncentral actions.

Evidence for CNS Control of HGP in the Human

Although the question of brain insulin action is difficult to address in the human, a recent study showed that oral administration of a K_{ATP} channel activator decreased HGP modestly (30%) in nondiabetic humans; however, inhibition was first apparent more than 5 hr after oral diazoxide consumption and was observed during a peripheral vein insulin clamp (Kishore et al., 2011). In rodent studies, hypothalamic K_{ATP} channel activation replicated the effect of ICV insulin administration on HGP, and coadministration of a K_{ATP} channel inhibitor blocked brain insulin action (Obici et al., 2002b; Pocai et al., 2005). Because activation of the K_{ATP} channel suppressed HGP in the human (Kishore et al., 2011), it raises the possibility that brain insulin action has the potential to regulate liver glucose metabolism in man, as it can in the rodent and the dog. It must be remembered, however, that the effect was slow to manifest and its physiologic relevance is unclear because, once again, the inhibition of HGP was observed during relative hepatic insulin deficiency. Further, whereas HGP responds to insulin within minutes in the human and because 5h after diazoxide consumption there was no effect on HGP, these data do not support the notion that acute changes in brain insulin action play a role in the rapid regulation of HGP.

Hepatic Denervation Does Not Adversely Affect Insulin-Mediated Control of HGP

Liver-transplanted (Lx) humans show no evidence of hepatic reinnervation for at least 30 months post-surgery (Kjaer et al., 1994). Thus, such patients can serve as a loss-of-function model, where hepatic glucose metabolism can be studied in the absence of any CNS input (including insulin-mediated input)

to the liver. Interpretation of data is complicated by immunosuppressive therapy in such patients, but this can be controlled for, at least in part, by comparison to carefully matched kidney-transplanted (Kx) patients or individuals with chronic uveitis (CU) who were maintained on similar treatment regimens.

Fasting plasma glucose and insulin levels of Lx patients were comparable to those of CU or Kx patients throughout the post-surgery follow-up period (up to 28 months) (Luzi et al., 1997; Perseghin et al., 1997; Schneiter et al., 2000a; Schneiter et al., 2000b). The suppression of HGP in Lx patients during hyperinsulinemic-hyperglycemic, euinsulinemic-hyperglycemic, and hyperinsulinemic-euglycemic clamps was similar to that observed in CU patients and healthy controls (Luzi et al., 1997; Perseghin et al., 1997). Following an oral glucose load, net hepatic glycogen synthesis (a correlate to hepatic glucose uptake) and the reduction in the rate of glucose appearance was similar in Lx and Kx patients compared to healthy controls studied 2–6 weeks after surgery (Schneiter et al., 2000b). The same Lx patients featured exaggerated postprandial glucose levels, relative to Kx patients, following an oral glucose load 38 weeks after surgery (Schneiter et al., 2000a), although this impaired glucose tolerance was likely due to relative circulating hypoinsulinemia (secondary to increased hepatic insulin clearance) rather than impaired hepatic insulin sensitivity. Thus, it appears that the regulation of HGP during fasting, insulin clamp, and postprandial conditions is essentially normal in the Lx patient. Consistent with this view, the incidence of new-onset diabetes following liver transplantation is similar to what occurs with other transplanted organs (Marchetti, 2004), suggesting that the total loss of neural input to the liver does not predispose the human to the disease. In support of these human data, the regulation of hepatic glucose metabolism during the basal state and also during hyperinsulinemic clamps and feeding conditions (i.e., mixed meal, oral glucose, or intraduodenal glucose challenges) was largely intact in dogs (Moore et al., 1993, 1994, 2002) and rats (Kissler et al., 2005) subjected to complete hepatic denervation.

It must be acknowledged, however, that neural input to the liver is complex. Hepatic innervation involves input from parasympathetic (cholinergic) and sympathetic (adrenergic) nerves, each carrying efferent and afferent fibers, as well as nonadrenergic/noncholinergic nerves (Yi et al., 2010). It is possible, for instance, that the lack of insulin-mediated vagal input to the liver (Pocai et al., 2005) is balanced and off-set by the lack of opposing sympathetic drive in Lx patients, resulting in normal insulin-mediated regulation of HGP. In support of this possibility is the finding that the daily oscillations in plasma glucose and hepatic glucoregulatory gene expression were normal in rats subjected to complete hepatic denervation, but aberrant in response to unilateral removal of either parasympathetic or sympathetic input to the liver (Cailotto et al., 2008). It is also possible that, in the absence of CNS insulin-mediated input to the liver, peripheral insulin-mediated mechanisms compensate in order to maintain normal hepatic glucose metabolism.

Further Considerations

Species differences. It should be noted that glucose metabolism is different in rodents and large animals in several regards. To begin with, basal rates of HGP after a short-term fast are 5- to 10-fold (per unit of body weight) greater in rodents than humans

or dogs. In addition, rodents deplete hepatic glycogen stores and, thus, the capacity for glycogenolysis, very quickly (within a few hours), whereas humans and dogs maintain hepatic glycogen, and significant glycogenolysis, even after 42 hr of fasting (Hendrick et al., 1990; Nuttall et al., 2008). The gluconeogenic pathway is, therefore, more critical for maintaining fasting HGP in the rodent than in large animals. This may be important because insulin action in the CNS is thought to suppress HGP in the rodent by inhibiting gluconeogenesis (Pocai et al., 2005). There are also species-dependent differences in hepatic innervation (Yi et al., 2010), and it may be that increased the gluconeogenic drive evident in rodents results in part from increased neural input.

Time course of CNS insulin action. Physiologic hyperinsulinemia rapidly (within minutes) suppresses HGP with marked inhibition evident within 1 hr (Ramnanan et al., 2010b). In addition, the meal-associated β -cell response typically peaks within 1 hr, with insulin secretion returning to basal within 3–4 hr as euglycemia is restored. Moreover, the gluconeogenic pathway is not suppressed by meal-associated hyperinsulinemia (Jin et al., 2003; Newgard et al., 1984; Nuttall et al., 2008), despite insulin's ability to substantially suppress PEPCK protein expression (Ramnanan et al., 2010b). Thus, it is unlikely that acute changes in brain insulin signaling, which appears to involve the genetic regulation of gluconeogenesis, would produce an effect on HGP during the time course of a meal. Instead, it would appear that the nongenomic (i.e., posttranslational) mechanisms are responsible for the rapid insulin-mediated suppression of HGP (Lin and Accili, 2011; Ramnanan et al., 2010a).

Although our canine studies do not support a role for acute changes in CNS insulin action in the rapid control of HGP, it is possible that long-term alteration of CNS insulin signaling may bring about important and sustained changes in hepatic glucoregulatory gene and protein expression, such that the response to increased insulin secretion, via direct or indirect mechanisms, would be altered. We examined the effects of brain insulin action on the rapid (0–4 hr) suppression of HGP in the dog when brain insulin signaling was modified for 1 hr prior to, or concurrently with, the insulin challenge. However, to our knowledge, all previous rodent studies have investigated the effects of systemic hyperinsulinemia for 90–120 min on the background of changes in brain insulin signaling that were in place for 4 hr to life (Guo et al., 2009; Hill et al., 2010; Inoue et al., 2006; Koch et al., 2008; Könnner et al., 2007; Lin et al., 2010; Obici et al., 2002a, 2002b; Ono et al., 2008; Park et al., 2009; Pocai et al., 2005). 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 (Hill et al., 2009; Park et al., 2009). It also appears that chronic hypothalamic insulin resistance may contribute to the development of hepatic insulin resistance (Hill et al., 2009, 2010; Koch et al., 2008; Könnner et al., 2007; Lin et al., 2010; Ono et al., 2008; Park et al., 2009). However, human type 2 diabetes has been shown to be associated with normal levels of hepatic G6Pase and PEPCK mRNA (Samuel et al., 2009), and it is currently unclear whether CNS insulin-regulated hepatic genes are aberrantly controlled in diabetic humans. It should also be noted that insulin has been shown to regulate food intake, energy homeostasis, and body fat mass (Guyenet

and Schwartz, 2012), and, thereby, chronic changes in brain insulin action may indirectly affect the response of the liver to the direct effects of insulin.

Postprandial conditions. Most studies to date have evaluated the impact of CNS insulin signaling on the liver under euglycemic conditions. In normal conditions, however, increased insulin secretion is associated with the response to nutrient ingestion. Thus, brain insulin action may be more relevant to the liver's response during times of nutrient excess (i.e., hyperglycemia). In line with this, brain insulin action in the dog alters the expression of genes important to glucose uptake (glucokinase) and glycogen synthesis (GSK3 β). In addition, it has been suggested that nutrient status may be important to brain insulin action in the rodent (Lin et al., 2010). Nevertheless, it should be noted that the suppression of HGP and the augmentation of net hepatic glucose uptake during the acute meal response occurs too quickly (within minutes) to involve genetic regulation unless the response to the first meal of the day sets the tone for the hepatic response to subsequent meals.

Alternatively, nongenomic effects of CNS insulin action may become manifest under feeding conditions when hyperglycemia exists. The delivery of glucose into the portal vein is known to amplify net hepatic glucose uptake, relative to glucose delivery into a peripheral vein, and this effect is manifest within minutes (Cherrington, 1999). The portal glucose signal controls the disposition of glucose between muscle and liver, reducing uptake by the former and enhancing uptake by the latter, without altering overall whole-body glucose tolerance. The enhancement of net hepatic glucose uptake by portal glucose delivery in the dog requires the presence of insulin (Pagliassotti et al., 1992), tends to increase with increasing levels of plasma insulin (Myers et al., 1991), and appears to be sensitive to hepatic denervation (Adkins-Marshall et al., 1992). Taking the above together, a role for CNS insulin sensing in regulating postprandial net hepatic glucose uptake cannot be ruled out. There is precedent for CNS insulin action bringing about peripheral effects rapidly, albeit not on the liver. For example, the sympathetic counter-regulatory response to hypoglycemia, which occurs within minutes and is responsible for the rapid life-saving rebound in blood glucose, was amplified by elevated insulin in human studies (Davis et al., 1993), an effect shown in rodents and dogs to be due to the impact of increased CNS insulin action (Davis et al., 1993, 1995; Diggs-Andrews et al., 2010; Fisher et al., 2005).

Glucagon. It must be noted that the impact of glucagon suppression is typically overlooked in studies evaluating CNS insulin's ability to suppress HGP. Rodent clamp studies evaluating CNS insulin action typically do not measure or consider the impact of the fall in plasma glucagon that is known to occur in response to the effect of hyperinsulinemia on the α -cell (Banarer et al., 2002; Kawamori et al., 2009). At the same time, our pancreatic clamp studies in dogs have determined the effects of CNS insulin action independent of the consequences of changes in glucagon secretion because it was clamped at a basal value. Recently, it was shown that local blockade of basal insulin action in the hypothalamus can increase glucagon secretion within minutes in rats (Paranjape et al., 2010). Likewise, hyperinsulinemia in dogs brought about by peripheral insulin infusion decreased both glucagon secretion and C-peptide

levels, indicative of decreased endogenous insulin secretion and, therefore, decreased intra-islet insulin concentrations (Cherrington, 2001). Thus, α -cell glucagon secretion was inhibited at a time when local insulin levels at the α -cell may have been reduced, raising the possibility that this effect was due to CNS insulin action. Evaluating the control of glucagon by insulin in the brain becomes especially problematic during meal-induced hyperinsulinemia, given the complexity of regulation of the α -cell by nutrients, hormones, and neural input, as well as autocrine and paracrine factors (Gromada et al., 2007). It remains to be determined whether CNS insulin-mediated control of glucagon secretion is of any significance to insulin's effects on HGP.

In summary, it is our view that, as with the rodent, the brain of large animals can sense physiologic changes in plasma insulin and relay that information to the liver and, perhaps, to other tissues. Data available to date suggest that acute changes in CNS insulin action bring about effects on hepatic gene transcription. If this is indeed the mechanism by which brain insulin action works, it would preclude its involvement in the rapid (<90 min) response of the liver to insulin. Indeed, both rodent and canine data indicate that the effect of brain insulin action on glucose flux requires several hours or more to be manifest. This, in turn, indicates that it is the noncentral effects of insulin on liver and fat that drive the quick response of the liver to the hormone. Further, rodent, dog, and human studies that have shown an effect of CNS action on liver glucose flux (at 2 hr or greater) have done so in the presence of relative hepatic insulin deficiency. In the presence of normally elevated arterial and hepatic insulin levels, where insulin levels at the liver are ~3-fold greater than at the brain, the central effects of insulin appear to be overridden by the hormone's direct effects on liver and fat. It is possible that the impact of CNS insulin action varies among species, given the differences in the basal metabolic rate and hepatic innervation. Indeed, in the rodent the impact of brain insulin action was gluconeogenic, whereas in the canine the effect was on glycogen metabolism. In addition, it should be remembered that chronic (hours to days) up- or downregulation of brain insulin action may very well affect the acute response of the liver to insulin by altering the baseline state of various glucoregulatory molecules. Finally, the role of CNS insulin action in response to nutrient intake still needs clarification. Thus, there is no doubt that the brain sensitively monitors the plasma insulin level and signals the liver. This mechanism may be involved in setting the basal tone for hepatic glucose metabolism, but there is no evidence to support a role for an acute change in brain insulin action in the rapid response of the liver to a rise in insulin secretion. The key need at present is to find out under which physiologic and pathologic conditions this regulatory mechanism is important.

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