Published in final edited form as:
Cell Metab. 2012 May 2; 15(5): 656–664. doi:10.1016/j.cmet.2012.03.006.

Evidence against a physiologic role for acute changes in CNS insulin action in the rapid regulation of hepatic glucose production

Christopher J. Ramnanan[1,2], **Dale S. Edgerton**[1], and **Alan D. Cherrington**[1]

[1]Vanderbilt University School of Medicine, Department of Molecular Physiology and Biophysics, Nashville, Tennessee, USA

[2]Medical College of Georgia at Georgia Health Sciences University, Department of Cellular Biology and Anatomy, Augusta, GA 30912

Abstract

This review will discuss the physiologic relevance of data which suggest that 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 while the canine brain can sense insulin, and thereby regulate hepatic glucoregulatory 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, as well as through indirect effects thought to be mediated primarily at adipose tissue and the α-cell (Fig. 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, to suppress HGP (Guo et al., 2009; Hill et al., 2010; Inoue et al., 2006; Koch et al., 2008; Konner et al., 2007; Lin et al., 2010; Obici et al., 2002a; Obici et al., 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 (Fig. 1). In addition, some studies of CNS insulin action have suggested that the central effect of insulin is required for the rapid suppression of HGP in

Address correspondence to: Dale Edgerton, Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232-0615, United States, Tel: 615-322-7014, Fax: 615-343-0490, dale.edgerton@vanderbilt.edu.

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 review 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 which 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 which indicate 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

While classic studies established that CNS insulin can regulate energy homeostasis (Woods et al., 1979) and pancreatic hormone secretion (Woods and Porte, 1975), there had been, until recently, 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 4^{th} 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 hour clamp (the 6^{th} 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 the suppression of HGP caused by the hyperinsulinemia resulting from peripheral vein infusion of insulin at 3.0 mU/kg/min for the last two hours 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 (Bruning et al., 2000; Koch et al., 2008; Lin et al., 2011), in the hypothalamus (Obici et al., 2002a; Ono et al., 2008), and in specific populations of hypothalamic neurons (Hill et al., 2010; Hill et al., 2009; Konner 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; Konner 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) (Konner 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., 2010; Hill et al., 2009; Koch et al., 2008; Konner 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 ~3-fold that of the insulin levels at non-hepatic 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 since endogenous insulin secretion is eliminated, marked hepatic insulin deficiency simultaneously occurs (Fig. 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 since 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 non-hepatic (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 in part due 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 the 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).

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 (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 non-physiologic 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 non-physiologic regionalization of the administered insulin. This is an important consideration, because while 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, since 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, since ICV delivery results in concentrations at ventricular surfaces which are likely to be greater than 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 which 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 non-hypothalamic sites did not contribute to the observed brain insulin response (Pocai et al., 2005). Third, PI3K is not only involved in insulin signaling, for example leptin also signals through this pathway (Hawkins et al., 2006; Warne et al.), 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 and glucagon into the hepatic portal vein and for the calculation of net hepatic glucose balance (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 (Fig. 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 non-hepatic 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, in order to bring about a selective ~3-fold increase in insulin at non-hepatic tissues, while maintaining basal insulin levels at the liver. Selective non-hepatic 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 non-hepatic hyperinsulinemia.

In another study, we assessed the relative importance of the direct (hepatic) and indirect (non-hepatic; 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 a ~2-fold increase in arterial insulin (ie. at non-hepatic tissues) and a ~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 non-hepatic (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 employed 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 rate 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 to suppress the mRNA expression of pyruvate carboxylase, G6Pase, and PEPCK by ~30–60% (Ramnanan et al., 2011a). Even though CSF insulin levels increased ~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. We therefore 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 4th 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 (Fig. 3). In accord with that, we observed that glucokinase mRNA was increased, and the mRNA and protein levels of GSK3β, another STAT3-regulated gene (Moh et al., 2008), were decreased by brain hyperinsulinemia. Reduced GSK3β protein was associated with decreased glycogen synthase phosphorylation, which resulted in enhanced glycogen synthase activity (Fig. 3). ICV administration of a PI3K inhibitor (LY294002) had no effect in the presence of basal insulin levels but it inhibited hypothalamic Akt phosphorylation and blocked the centrally mediated downstream molecular consequences of insulin in the liver, as well as the associated changes in glucose kinetics.

The above studies (Ramnanan et al., 2011a), and previous studies in rodents, all defined brain insulin's effects in the context of relative hepatic insulin deficiency (Fig. 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 GSK3β expression, and the induction of glucokinase expression was under dual brain and hepatic insulin-mediated regulation. Non-genomic 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 non-central 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 (inhibition was first apparent, however, more than 5 hours 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 co-administration of a K_{ATP} channel inhibitor blocked brain insulin action (Obici et al., 2002b; Pocai et al., 2005). Since 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 does in the rodent and the dog. It must be remembered, however, that the effect was slow to manifest and its physiologic relevance is unclear, since once again the inhibition of HGP was observed during relative hepatic insulin deficiency. Further, since HGP responds to insulin within minutes in the human, and since 5h after diazoxide consumption there was no effect on HGP, these data support the notion that acute changes in brain insulin action do not 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) to the liver. Interpretation of data is complicated by immunosuppressive therapy in such patients, but this can be controlled for, in part at least, 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 patients 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, during hyperinsulinemic clamps and during feeding conditions (ie. mixed meal, oral glucose or intraduodenal glucose challenges) was largely intact in dogs (Moore et al., 1994; Moore et al., 2002; Moore et al., 1993) 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 non-adrenergic/non-cholinergic 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 5-10-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 42h 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 since 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 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 1h (Ramnanan et al., 2010b). In addition, the meal-associated β-cell response typically peaks within 1h, with insulin secretion returning to basal within 3–4h 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 appear 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 non-genomic (post-translational) mechanisms are responsible for the rapid insulin-mediated suppression of HGP (Lin and Accili, 2011; Ramnanan et al., 2010a).

While 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 to 4h) suppression of HGP in the dog when brain insulin signaling was modified for 1h 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 to 120 min, and on the background of changes in brain insulin signaling that were in place for 4h to life (Guo et al., 2009; Hill et al., 2010; Inoue et al., 2006; Koch et al., 2008; Konner et al., 2007; Lin et al., 2010; Obici et al., 2002a; Obici et al., 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., 2010; Hill et al., 2009; Koch et al., 2008; Konner et al., 2007; Lin et al., 2010; Ono et al., 2008; Park et al., 2009). Human type 2 diabetes has been shown to be associated with normal levels of hepatic G6Pase and PEPCK mRNA (Samuel et al., 2009) however, 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 (hyperglycemia). In line with this, brain insulin action in the dog alters the expression of genes important to glucose uptake (glucokinase) and glycogen synthesis (GSK3β). 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, non-genomic 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., 1995; Davis et al., 1993; 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 since it was clamped at a basal value. Recently it was shown that the 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 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 non-central effects of insulin on liver and fat which drive the quick response of the liver to the hormone. Further, rodent, dog and human studies which have shown an effect of CNS insulin action on liver glucose flux (at 2h 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 will vary with the species, given the differences in the basal metabolic rate and hepatic innervation. Indeed, in the rodent the impact of brain insulin action was gluconeogenic, while in the canine the effect was on glycogen metabolism. In addition, it should be remembered that chronic (hours to days) up- or down-regulation 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 to be clarified. 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.

Acknowledgments

This research was supported in part by the NIH Grant R01-DK-18243 and the DRTC Grant SP-60-AM20593. CJR was supported by the American Diabetes Association Mentor-based Fellowship. ADC was supported by the Jacquelyn A. Turner and Dr. Dorothy J. Turner Chair in Diabetes Research.

References

Adkins-Marshall B, Pagliassotti MJ, Asher JR, Connolly CC, Neal DW, Williams PE, Myers SR, Hendrick GK, Adkins RB Jr, Cherrington AD. Role of hepatic nerves in response of liver to intraportal glucose delivery in dogs. Am J Physiol. 1992; 262:E679–686. [PubMed: 1590377]

Banarer S, McGregor VP, Cryer PE. Intraislet hyperinsulinemia prevents the glucagon response to hypoglycemia despite an intact autonomic response. Diabetes. 2002; 51:958–965. [PubMed: 11916913]

Bergman RN. New concepts in extracellular signaling for insulin action: the single gateway hypothesis. Recent Prog Horm Res. 1997; 52:359–385. discussion 385–357. [PubMed: 9238859]

Brand CL, Jorgensen PN, Knigge U, Warberg J, Svendsen I, Kristensen JS, Holst JJ. Role of glucagon in maintenance of euglycemia in fed and fasted rats. Am J Physiol. 1995; 269:E469–477. [PubMed: 7573424]

Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, Kahn CR. Role of brain insulin receptor in control of body weight and reproduction. Science. 2000; 289:2122–2125. [PubMed: 11000114]

Cailotto C, van Heijningen C, van der Vliet J, van der Plasse G, Habold C, Kalsbeek A, Pevet P, Buijs RM. Daily rhythms in metabolic liver enzymes and plasma glucose require a balance in the autonomic output to the liver. Endocrinology. 2008; 149:1914–1925. [PubMed: 18162516]

Cherrington AD. Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. Diabetes. 1999; 48:1198–1214. [PubMed: 10331429]

Cherrington, AD. Control of Glucose Production in vivo by insulin and glucagon. In: LSC; Jefferson, AD., editors. Handbook of Physiology. New York: Oxford University Press; 2001. p. 759-785.

Cherrington AD, Chiasson JL, Liljenquist JE, Lacy WW, Park CR. Control of hepatic glucose output by glucagon and insulin in the intact dog. Biochem Soc Symp. 1978:31–45. [PubMed: 373768]

Chu CA, Fujimoto Y, Igawa K, Grimsby J, Grippo JF, Magnuson MA, Cherrington AD, Shiota M. Rapid translocation of hepatic glucokinase in response to intraduodenal glucose infusion and changes in plasma glucose and insulin in conscious rats. Am J Physiol Gastrointest Liver Physiol. 2004; 286:G627–634. [PubMed: 14656711]

Davis SN, Colburn C, Dobbins R, Nadeau S, Neal D, Williams P, Cherrington AD. Evidence that the brain of the conscious dog is insulin sensitive. J Clin Invest. 1995; 95:593–602. [PubMed: 7860743]

Davis SN, Goldstein RE, Jacobs J, Price L, Wolfe R, Cherrington AD. The effects of differing insulin levels on the hormonal and metabolic response to equivalent hypoglycemia in normal humans. Diabetes. 1993; 42:263–272. [PubMed: 8425662]

Diggs-Andrews KA, Zhang X, Song Z, Daphna-Iken D, Routh VH, Fisher SJ. Brain insulin action regulates hypothalamic glucose sensing and the counterregulatory response to hypoglycemia. Diabetes. 2010; 59:2271–2280. [PubMed: 20547974]

Edgerton DS, Lautz M, Scott M, Everett CA, Stettler KM, Neal DW, Chu CA, Cherrington AD. Insulin's direct effects on the liver dominate the control of hepatic glucose production. J Clin Invest. 2006; 116:521–527. [PubMed: 16453026]

Edgerton DS, Ramnanan CJ, Grueter CA, Johnson KM, Lautz M, Neal DW, Williams PE, Cherrington AD. Effects of insulin on the metabolic control of hepatic gluconeogenesis in vivo. Diabetes. 2009a; 58:2766–2775. [PubMed: 19755527]

Edgerton DS, Ramnanan CJ, Williams P, Farmer T, Neal D, Donahue P, Cherrington AD. Acute inhibition of hepatic glucose production by insulin does not depend on insulin signaling and action in the brain of the conscious dog. Diabetes. 2009b; 58:A80.

Fisher SJ, Bruning JC, Lannon S, Kahn CR. Insulin signaling in the central nervous system is critical for the normal sympathoadrenal response to hypoglycemia. Diabetes. 2005; 54:1447–1451. [PubMed: 15855332]

Gelling RW, Morton GJ, Morrison CD, Niswender KD, Myers MG Jr, Rhodes CJ, Schwartz MW. Insulin action in the brain contributes to glucose lowering during insulin treatment of diabetes. Cell Metab. 2006; 3:67–73. [PubMed: 16399506]

Gharbi SI, Zvelebil MJ, Shuttleworth SJ, Hancox T, Saghir N, Timms JF, Waterfield MD. Exploring the specificity of the PI3K family inhibitor LY294002. Biochem J. 2007; 404:15–21. [PubMed: 17302559]

Gromada J, Franklin I, Wollheim CB. Alpha-cells of the endocrine pancreas: 35 years of research but the enigma remains. Endocr Rev. 2007; 28:84–116. [PubMed: 17261637]

Guo S, Copps KD, Dong X, Park S, Cheng Z, Pocai A, Rossetti L, Sajan M, Farese RV, White MF. The Irs1 branch of the insulin signaling cascade plays a dominant role in hepatic nutrient homeostasis. Mol Cell Biol. 2009; 29:5070–5083. [PubMed: 19596788]

Guyenet SJ, Schwartz MW. Regulation of Food Intake, Energy Balance, and Body Fat Mass: Implications for the Pathogenesis and Treatment of Obesity. J Clin Endocrinol Metab. 201210.1210/jc.2011–2525

Hawkins PT, Anderson KE, Davidson K, Stephens LR. Signalling through Class I PI3Ks in mammalian cells. Biochem Soc Trans. 2006; 34:647–662. [PubMed: 17052169]

Hendrick GK, Frizzell RT, Williams PE, Cherrington AD. Effect of hyperglucagonemia on hepatic glycogenolysis and gluconeogenesis after a prolonged fast. Am J Physiol. 1990; 258:E841–849. [PubMed: 2185665]

Hill JW, Elias CF, Fukuda M, Williams KW, Berglund ED, Holland WL, Cho YR, Chuang JC, Xu Y, Choi M, Lauzon D, Lee CE, Coppari R, Richardson JA, Zigman JM, Chua S, Scherer PE, Lowell BB, Bruning JC, Elmquist JK. Direct insulin and leptin action on pro-opiomelanocortin neurons is required for normal glucose homeostasis and fertility. Cell Metab. 2010; 11:286–297. [PubMed: 20374961]

Hill JW, Xu Y, Preitner F, Fukuda M, Cho YR, Luo J, Balthasar N, Coppari R, Cantley LC, Kahn BB, Zhao JJ, Elmquist JK. Phosphatidyl inositol 3-kinase signaling in hypothalamic proopiomelanocortin neurons contributes to the regulation of glucose homeostasis. Endocrinology. 2009; 150:4874–4882. [PubMed: 19819947]

Horwitz DL, Starr JI, Mako ME, Blackard WG, Rubenstein AH. Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood. J Clin Invest. 1975; 55:1278–1283. [PubMed: 1133173]

Inoue H, Ogawa W, Asakawa A, Okamoto Y, Nishizawa A, Matsumoto M, Teshigawara K, Matsuki Y, Watanabe E, Hiramatsu R, Notohara K, Katayose K, Okamura H, Kahn CR, Noda T, Takeda K, Akira S, Inui A, Kasuga M. Role of hepatic STAT3 in brain-insulin action on hepatic glucose production. Cell Metab. 2006; 3:267–275. [PubMed: 16581004]

Jin ES, Uyeda K, Kawaguchi T, Burgess SC, Malloy CR, Sherry AD. Increased hepatic fructose 2,6-bisphosphate after an oral glucose load does not affect gluconeogenesis. J Biol Chem. 2003; 278:28427–28433. [PubMed: 12764148]

Kawamori D, Kurpad AJ, Hu J, Liew CW, Shih JL, Ford EL, Herrera PL, Polonsky KS, McGuinness OP, Kulkarni RN. Insulin signaling in alpha cells modulates glucagon secretion in vivo. Cell Metab. 2009; 9:350–361. [PubMed: 19356716]

Kishore P, Boucai L, Zhang K, Li W, Koppaka S, Kehlenbrink S, Schiwek A, Esterson YB, Mehta D, Bursheh S, Su Y, Gutierrez-Juarez R, Muzumdar R, Schwartz GJ, Hawkins M. Activation of KATP channels suppresses glucose production in humans. J Clin Invest. 2011; 121:4916–4920. [PubMed: 22056385]

Kissler HJ, Hauffen J, Hennig R, Gepp H, Schwille PO. Glucose and lipid metabolism after liver transplantation in inbred rats: consequences of hepatic denervation. Metabolism. 2005; 54:881–890. [PubMed: 15988696]

Kjaer M, Jurlander J, Keiding S, Galbo H, Kirkegaard P, Hage E. No reinnervation of hepatic sympathetic nerves after liver transplantation in human subjects. J Hepatol. 1994; 20:97–100. [PubMed: 8201229]

Koch L, Wunderlich FT, Seibler J, Konner AC, Hampel B, Irlenbusch S, Brabant G, Kahn CR, Schwenk F, Bruning JC. Central insulin action regulates peripheral glucose and fat metabolism in mice. J Clin Invest. 2008; 118:2132–2147. [PubMed: 18451994]

Konner AC, Janoschek R, Plum L, Jordan SD, Rother E, Ma X, Xu C, Enriori P, Hampel B, Barsh GS, Kahn CR, Cowley MA, Ashcroft FM, Bruning JC. Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production. Cell Metab. 2007; 5:438–449. [PubMed: 17550779]

Levin BE, Sherwin RS. Peripheral glucose homeostasis: does brain insulin matter? J Clin Invest. 2011

Li JH, Gautam D, Han SJ, Guettier JM, Cui Y, Lu H, Deng C, O'Hare J, Jou W, Gavrilova O, Buettner C, Wess J. Hepatic muscarinic acetylcholine receptors are not critically involved in maintaining glucose homeostasis in mice. Diabetes. 2009; 58:2776–2787. [PubMed: 19752163]

Liljenquist JE, Mueller GL, Cherrington AD, Keller U, Chiasson JL, Perry JM, Lacy WW, Rabinowitz D. Evidence for an important role of glucagon in the regulation of hepatic glucose production in normal man. J Clin Invest. 1977; 59:369–374. [PubMed: 833282]

Lin HV, Accili D. Hormonal regulation of hepatic glucose production in health and disease. Cell Metab. 2011; 14:9–19. [PubMed: 21723500]

Lin HV, Plum L, Ono H, Gutierrez-Juarez R, Shanabrough M, Borok E, Horvath TL, Rossetti L, Accili D. Divergent regulation of energy expenditure and hepatic glucose production by insulin receptor in agouti-related protein and POMC neurons. Diabetes. 2010; 59:337–346. [PubMed: 19933998]

Lin HV, Ren H, Samuel VT, Lee HY, Lu TY, Shulman GI, Accili D. Diabetes in mice with selective impairment of insulin action in Glut4-expressing tissues. Diabetes. 2011; 60:700–709. [PubMed: 21266328]

Luzi L, Perseghin G, Regalia E, Sereni LP, Battezzati A, Baratti D, Bianchi E, Terruzzi I, Hilden H, Groop LC, Pulvirenti A, Taskinen MR, Gennari L, Mazzaferro V. Metabolic effects of liver transplantation in cirrhotic patients. J Clin Invest. 1997; 99:692–700. [PubMed: 9045872]

Marchetti P. New-onset diabetes after transplantation. J Heart Lung Transplant. 2004; 23:S194–201. [PubMed: 15093805]

Moh A, Zhang W, Yu S, Wang J, Xu X, Li J, Fu XY. STAT3 sensitizes insulin signaling by negatively regulating glycogen synthase kinase-3 beta. Diabetes. 2008; 57:1227–1235. [PubMed: 18268048]

Moore MC, Pagliassotti MJ, Goldstein RE, Asher J, Murrell J, Neal D, Cherrington AD. The effect of the hepatic nerves on the disposition of a mixed meal by conscious dogs. JPEN J Parenter Enteral Nutr. 1994; 18:248–255. [PubMed: 8065001]

Moore MC, Satake S, Baranowski B, Hsieh PS, Neal DW, Cherrington AD. Effect of hepatic denervation on peripheral insulin sensitivity in conscious dogs. Am J Physiol Endocrinol Metab. 2002; 282:E286–296. [PubMed: 11788359]

Moore MC, Shulman GI, Giaccari A, Pagliassotti MJ, Cline G, Neal D, Rossetti L, Cherrington AD. Effect of hepatic nerves on disposition of an intraduodenal glucose load. Am J Physiol. 1993; 265:E487–496. [PubMed: 8214056]

Myers SR, McGuinness OP, Neal DW, Cherrington AD. Intraportal glucose delivery alters the relationship between net hepatic glucose uptake and the insulin concentration. J Clin Invest. 1991; 87:930–939. [PubMed: 1999501]

Newgard CB, Moore SV, Foster DW, McGarry JD. Efficient hepatic glycogen synthesis in refeeding rats requires continued carbon flow through the gluconeogenic pathway. J Biol Chem. 1984; 259:6958–6963. [PubMed: 6725277]

Nuttall FQ, Ngo A, Gannon MC. Regulation of hepatic glucose production and the role of gluconeogenesis in humans: is the rate of gluconeogenesis constant? Diabetes Metab Res Rev. 2008; 24:438–458. [PubMed: 18561209]

Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. Nat Neurosci. 2002a; 5:566–572. [PubMed: 12021765]

Obici S, Zhang BB, Karkanias G, Rossetti L. Hypothalamic insulin signaling is required for inhibition of glucose production. Nat Med. 2002b; 8:1376–1382. [PubMed: 12426561]

Ono H, Pocai A, Wang Y, Sakoda H, Asano T, Backer JM, Schwartz GJ, Rossetti L. Activation of hypothalamic S6 kinase mediates diet-induced hepatic insulin resistance in rats. J Clin Invest. 2008; 118:2959–2968. [PubMed: 18618016]

Pagliassotti MJ, Moore MC, Neal DW, Cherrington AD. Insulin is required for the liver to respond to intraportal glucose delivery in the conscious dog. Diabetes. 1992; 41:1247–1256. [PubMed: 1397697]

Paranjape SA, Chan O, Zhu W, Horblitt AM, McNay EC, Cresswell JA, Bogan JS, McCrimmon RJ, Sherwin RS. Influence of insulin in the ventromedial hypothalamus on pancreatic glucagon secretion in vivo. Diabetes. 2010; 59:1521–1527. [PubMed: 20299468]

Park S, Hong SM, Ahn IS. Long-term intracerebroventricular infusion of insulin, but not glucose, modulates body weight and hepatic insulin sensitivity by modifying the hypothalamic insulin signaling pathway in type 2 diabetic rats. Neuroendocrinology. 2009; 89:387–399. [PubMed: 19176946]

Perseghin G, Regalia E, Battezzati A, Vergani S, Pulvirenti A, Terruzzi I, Baratti D, Bozzetti F, Mazzaferro V, Luzi L. Regulation of glucose homeostasis in humans with denervated livers. J Clin Invest. 1997; 100:931–941. [PubMed: 9259593]

Pocai A, Lam TK, Gutierrez-Juarez R, Obici S, Schwartz GJ, Bryan J, Aguilar-Bryan L, Rossetti L. Hypothalamic K(ATP) channels control hepatic glucose production. Nature. 2005; 434:1026–1031. [PubMed: 15846348]

Ramnanan CJ, Edgerton DS, Cherrington AD. The Role of Insulin in the Regulation of PEPCK and Gluconeogenesis In Vivo. US Endocrinology. 2010a; 5:34–39.

Ramnanan CJ, Edgerton DS, Rivera N, Irimia-Dominguez J, Farmer B, Neal DW, Lautz M, Donahue EP, Meyer CM, Roach PJ, Cherrington AD. Molecular characterization of insulin-mediated suppression of hepatic glucose production in vivo. Diabetes. 2010b; 59:1302–1311. [PubMed: 20185816]

Ramnanan CJ, Saraswathi V, Smith MS, Donahue EP, Farmer B, Farmer TD, Neal D, Williams PE, Lautz M, Mari A, Cherrington AD, Edgerton DS. Brain insulin action augments hepatic glycogen synthesis without suppressing glucose production or gluconeogenesis in dogs. J Clin Invest. 2011a; 121:3713–3723. [PubMed: 21865644]

Ramnanan CJ, Smith M, Donahue P, Farmer B, Neal D, Williams P, Lautz M, Snead W, Edgerton DS, Cherrington AD. Regulation of hepatic glucose metabolism by brain insulin action. Diabetes. 2011b; 60:A123.

Rebrin K, Steil GM, Mittelman SD, Bergman RN. Causal linkage between insulin suppression of lipolysis and suppression of liver glucose output in dogs. J Clin Invest. 1996; 98:741–749. [PubMed: 8698866]

Rizza RA. Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: implications for therapy. Diabetes. 2010; 59:2697–2707. [PubMed: 20705776]

Samuel VT, Beddow SA, Iwasaki T, Zhang XM, Chu X, Still CD, Gerhard GS, Shulman GI. Fasting hyperglycemia is not associated with increased expression of PEPCK or G6Pc in patients with Type 2 Diabetes. Proc Natl Acad Sci U S A. 2009

Sandoval DA, Obici S, Seeley RJ. Targeting the CNS to treat type 2 diabetes. Nat Rev Drug Discov. 2009; 8:386–398. [PubMed: 19404312]

Scherer T, O'Hare J, Diggs-Andrews K, Schweiger M, Cheng B, Lindtner C, Zielinski E, Vempati P, Su K, Dighe S, Milsom T, Puchowicz M, Scheja L, Zechner R, Fisher SJ, Previs SF, Buettner C. Brain insulin controls adipose tissue lipolysis and lipogenesis. Cell Metab. 2011; 13:183–194. [PubMed: 21284985]

Schneiter P, Gillet M, Chiolero R, Jequier E, Mosimann F, Temler E, Teta D, Matter M, Wauters JP, Tappy L. Mechanisms of postprandial hyperglycemia in liver transplant recipients: comparison of liver transplant patients with kidney transplant patients and healthy controls. Diabetes Metab. 2000a; 26:51–56. [PubMed: 10705104]

Schneiter P, Gillet M, Chiolero R, Wauters JP, Berger M, Tappy L. Postprandial hepatic glycogen synthesis in liver transplant recipients. Transplantation. 2000b; 69:978–981. [PubMed: 10755560]

Sindelar DK, Chu CA, Rohlie M, Neal DW, Swift LL, Cherrington AD. The role of fatty acids in mediating the effects of peripheral insulin on hepatic glucose production in the conscious dog. Diabetes. 1997; 46:187–196. [PubMed: 9000693]

Warne JP, Alemi F, Reed AS, Varonin JM, Chan H, Piper ML, Mullin ME, Myers MG Jr, Corvera CU, Xu AW. Impairment of Central Leptin-Mediated PI3K Signaling Manifested as Hepatic Steatosis Independent of Hyperphagia and Obesity. Cell Metab. 14:791–803. [PubMed: 22152304]

Woods SC, Lotter EC, McKay LD, Porte D Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. Nature. 1979; 282:503–505. [PubMed: 116135]

Woods SC, Porte D Jr. Effect of intracisternal insulin on plasma glucose and insulin in the dog. Diabetes. 1975; 24:905–909. [PubMed: 1100459]

Yi CX, la Fleur SE, Fliers E, Kalsbeek A. The role of the autonomic nervous liver innervation in the control of energy metabolism. Biochim Biophys Acta. 2010; 1802:416–431. [PubMed: 20060897]

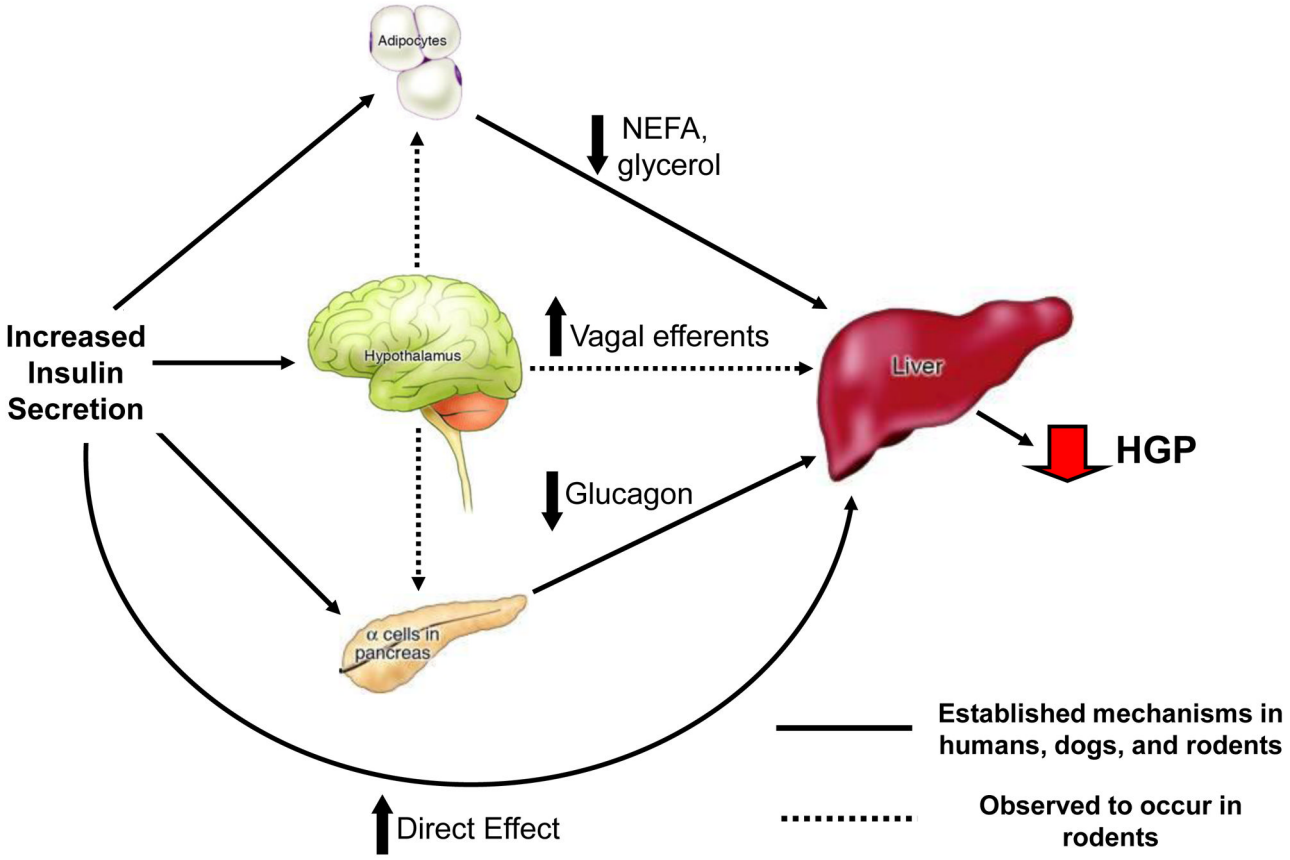

Fig 1.

The control of hepatic glucose production (HGP) by insulin. Insulin suppresses HGP by both direct effects (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.

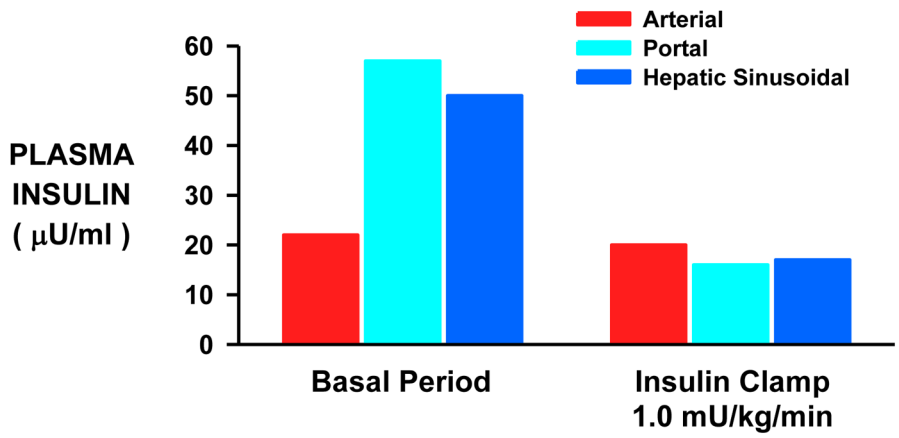

Fig 2.

Hepatic and non-hepatic 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 which result from endogenous secretion or peripheral vein infusion (Chu et al., 2004; Moore et al., 2002). Because of high 1st pass insulin clearance hepatic insulin levels are ~3-fold greater than concentrations in the artery. During the clamp, somatostatin is infused and non-hepatic (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.

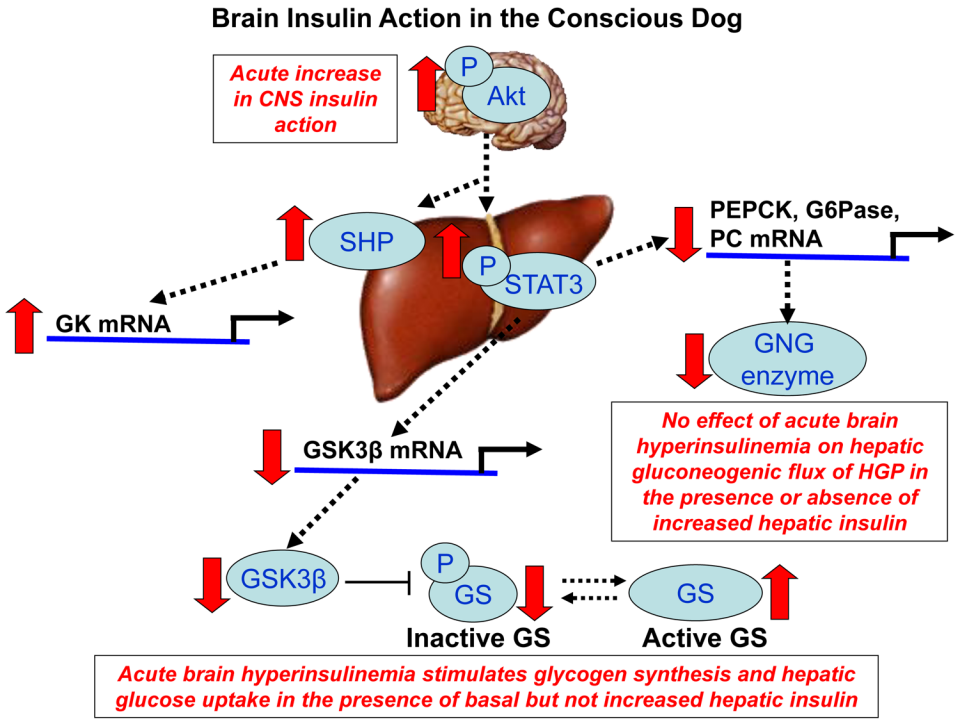

Fig 3.

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 (GSK3β) 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 GSK3β. During physiologic hyperinsulinemia brain insulin action does not impact the rapid suppression of hepatic glucose metabolism.