Metabolism

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Insulin action on the liver *in vivo*

A.D. Cherrington[1], M.C. Moore, D.K. Sindelar and D.S. Edgerton
Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, 704 Robinson Research Bldg, Nashville, TN 37232-0615, U.S.A.

Abstract

Insulin has a potent inhibitory effect on hepatic glucose production by direct action at hepatic receptors. The hormone also inhibits glucose production by suppressing both lipolysis in the fat cell and secretion of glucagon by the α-cell. Neural sensing of insulin levels appears to participate in control of hepatic glucose production in rodents, but a role for brain insulin sensing has not been documented in dogs or humans. The primary effect of insulin on the liver is its direct action.

Introduction

Insulin is well recognized as a potent inhibitor of hepatic glucose production. It is this action, along with its ability to augment glucose disposal, that makes it the primary glucoregulatory hormone *in vivo*. *In vivo* insulin action at the liver is countered by the action of glucagon, which stimulates hepatic glucose production. It is the dual control of the liver by these two hormones that allows precise regulation of hepatic glucose output. In the present paper, we examine the physiological mechanisms by which insulin inhibits hepatic glucose production *in vivo*.

Direct actions of insulin

The pancreatic clamp has been widely used to understand the regulation of glucose production *in vivo*. It involves the infusion of somatostatin to inhibit the endocrine pancreas and the simultaneous replacement of basal insulin and glucagon secretion by infusion. In the dog, these infusions can be given directly into the hepatic portal vein, thus allowing replacement of insulin and glucagon not only at their normal secretory rate but also at their normal site of entry into the circulation. In this way, basal levels of insulin and glucagon can be maintained throughout the body without altering glucose metabolism, but with a loss of feedback control of the pancreas [1]. Such an approach was used a number of years ago to define the ability of insulin itself (i.e. with glucagon kept basal) to inhibit glucose production *in vivo*. Studies showed that, after an overnight fast, basal insulin restrains glucose production by more than 50% (Figure 1) [1]. Likewise, the data in Figure 1 show that a tripling of insulin secretion (i.e. a 3-fold rise in plasma insulin) completely inhibited hepatic glucose output regardless of whether the studies were carried out in the dog or human [2–4]. Further analysis indicated that the acute inhibitory effect of insulin on glucose output by the liver primarily reflected an inhibition of glycogenolysis. Little if any change occurred in gluconeogenesis [1], at least within a 3 h observation period.

Indirect actions of insulin: lipolysis

Work by Prager et al. [5] and others [6,7] raised the possibility that insulin's effect on the liver occurred not only by direct interaction with the hepatic insulin receptor but also via an indirect effect resulting from insulin action on other tissues. We examined this question in the dog by placing infusion catheters in a peripheral vein and the hepatic portal vein. We were then able to adjust the insulin infusion rate at each site during a pancreatic clamp so as to bring about a selective rise in either hepatic portal vein insulin (i.e. arterial insulin basal and fixed) or in arterial insulin (i.e. hepatic portal vein insulin basal and fixed). A selective 14 μ-units/ml rise in hepatic portal vein insulin inhibited net hepatic glucose output rapidly by $\sim$50% [8]. A rise in arterial insulin of the same magnitude caused a similar inhibition of hepatic glucose output but required a longer time to do so. In other studies, a selective lowering of portal vein insulin (i.e. arterial insulin basal and fixed) caused a marked, rapid and sustained increase in glucose output by the liver [9]. Once again, this effect was solely due to a modification of glycogenolysis. We [10] and others [11] went on to show that the indirect effect of insulin on the liver seen in the above experiments resulted

Key words: glucagon, hyperglycaemia, insulin, intralipid, liver, somatostatin.
Abbreviations used: IR-AS, insulin receptor antisense; NEFA, non-esterified fatty acid; PI3K, phosphoinositide 3-kinase.
[1]To whom correspondence should be addressed (email alan.cherrington@vanderbilt.edu).

Figure 1 | Dose–response curves relating estimated hepatic sinusoidal insulin levels to net hepatic glucose output in the dog or glucose production in the human

Data taken from [2–4].

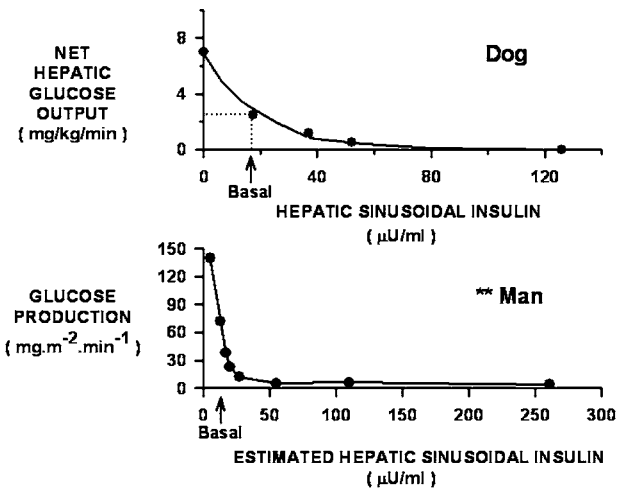
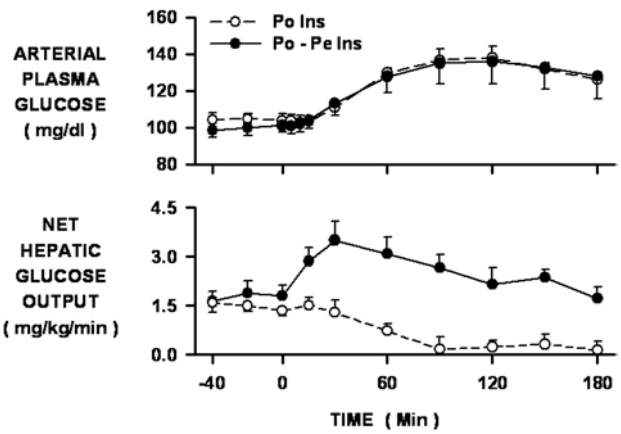


Figure 2 | Effects of direct and indirect insulin infusion in two groups of overnight-fasted dogs

At $t = 0$, the basal portal insulin infusion was continued (Po Ins; ○) or the portal insulin infusion was stopped and an infusion of insulin at the same rate was started in a leg vein (Po-Pe Ins; ●). As a result, the arterial plasma insulin nearly doubled, while the hepatic portal vein insulin level halved. Glucose was infused in the portal insulin group to match the glycaemia in the peripheral insulin group. An increase in net hepatic glucose output occurred coincident with the switch in the insulin infusion site, which was significant ($P < 0.05$) over the entire 3 h observation period. Data taken from [12].



from an inhibition of lipolysis. When we repeated our study in which the arterial insulin level was selectively increased by 14 μ-units/ml, but did so in the presence of an infusion of intralipid and heparin to clamp the arterial plasma NEFA (non-esterified fatty acid) level at basal values, the inhibition of hepatic glucose output was almost eliminated. Further, we were able to show that the decrease in plasma NEFA in the absence of intralipid caused a diversion of glycogenolytically derived glucose 6-phosphate from export as glucose to export as lactate following increased glycolytic flux.

In addition to the above effects, a selective rise in arterial insulin caused a modest decrease in the flux of gluconeogenic amino acids and glycerol to the liver, which in turn caused a small decrease in gluconeogenesis. The impact of this on the fall in glucose production was minimal, however, when compared with the impact of the redirection of glucose 6-phosphate down the glycolytic pathway.

Next, the question arises as to which of these effects on the liver (direct or indirect) is dominant. To answer this question, we carried out studies in which we again used a pancreatic clamp. Initially, we took over control of the endocrine pancreas in the conscious dog using portal insulin infusion. After a basal period, we switched the site of insulin infusion from the hepatic portal vein to a leg vein. As expected, the arterial insulin level almost doubled, whereas the hepatic portal vein insulin level was reduced by ~50% [12]. Glucose production immediately increased and hyperglycaemia resulted (Figure 2). In the control group, the route of insulin delivery was not changed, but glucose was infused to match the glycaemia to that in the peripheral insulin infusion group. As a result, hepatic glucose output fell over time due to the well-known inhibitory effect of hyperglycaemia on hepatic glucose production. Despite a near doubling of the arterial insulin level, the fall in insulin within the liver sinusoids resulted in an overproduction of glucose, which was evident for the full 3 h of study. These results clearly established the dominance of insulin's direct effect on the liver.

Indirect actions of insulin: pancreas and the nervous system

In addition to insulin's suppressive effects on lipolysis and proteolysis, two other non-hepatic effects of insulin have been reported to inhibit hepatic glucose output. The α-cell is insulin-sensitive, such that increments in plasma insulin will inhibit glucagon secretion [2]. Since glucagon is a potent regulator of liver glucose output, this would trigger a reduction in glucose production. In addition, Rossetti and co-workers [13,14] have recently suggested that the brain is sensitive to the plasma insulin level, and thus insulin action in the brain may also explain part of insulin's indirect action on the liver. Rossetti and co-workers infused insulin into the third ventricle of the rat for a total of 6 h. In the fourth hour, there was no change in glucose production. At that point, they began a pancreatic clamp to fix the circulating insulin level at a basal value. In the sixth hour of the study, they again assessed glucose turnover. In the control group, glucose production had fallen by ~1.8 $mg \cdot kg^{-1} \cdot min^{-1}$ (14%) from the 4 h point, while in the intracerebroventricularly injected insulin group it had fallen by 6.0 $mg \cdot kg^{-1} \cdot min^{-1}$ (43%). Thus they concluded that hypothalamic insulin signalling could be important to the action of insulin on the liver.

Certain issues should be kept in mind when extrapolating the conclusion of Rossetti and co-workers [13,14] to the acute

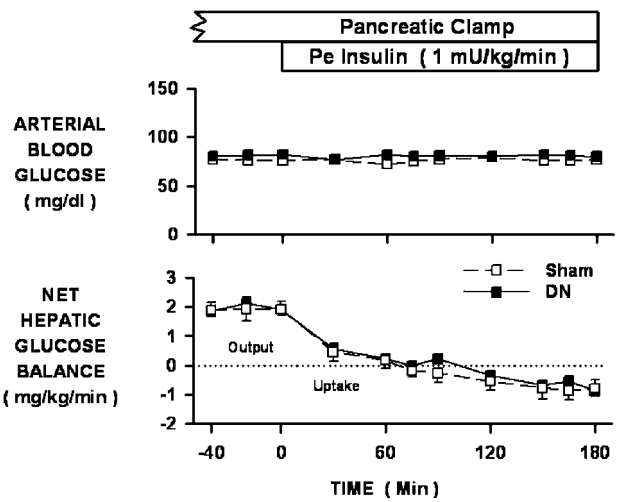
effects of elevated plasma insulin on hepatic glucose output in a large animal such as the dog or human. First, the effect of insulin given into the third ventricle or directly into the hypothalamus on hepatic glucose output was slow (hours) to develop [13], while the acute effect of a rise in plasma insulin on the glucose production in the dog and human occurs in minutes. Secondly, the inhibition of hepatic glucose production by a rise in brain insulin was small (~30%), and it is unclear whether higher insulin levels would have inhibited it further [13]. Thirdly, the relevance of the insulin level employed is unclear, but it was far in excess of levels that would ever exist in the cerebrospinal fluid *in vivo*. Finally, the glucose production rate of a rat ($\sim 12\ mg \cdot kg^{-1} \cdot min^{-1}$) or mouse ($\sim 20\ mg \cdot kg^{-1} \cdot min^{-1}$) is much greater than that of the dog or human (~ 2–$3\ mg \cdot kg^{-1} \cdot min^{-1}$) [13]. If the higher glucose production rate is explained by a higher level of neural input to the liver in the rodent, one might expect to see a brain-driven inhibition of glucose production in the rat or mouse but not in the dog or human.

In other experiments, Obici et al. [13] infused an IR-AS (insulin receptor antisense) oligonucleotide or an inhibitor of PI3K (phosphoinositide 3-kinase) into the third ventricle of rats for 6 h. Tracer-determined glucose production was measured between 3.5 and 4 h, and altering insulin signalling in the brain was without consequences. The systemic plasma insulin level was then raised by intravenous infusion for 2 h and the plasma glucose level was clamped at a euglycaemic value. The ability of the systemic rise in insulin to impair hepatic glucose production in the presence of these inhibitors of insulin action was then assessed. In the control group, the elevation in insulin decreased glucose production by $\sim 6.4\ mg \cdot kg^{-1} \cdot min^{-1}$. In the presence of the IR-AS, the rise in insulin inhibited glucose production by $\sim 4.0\ mg \cdot kg^{-1} \cdot min^{-1}$, while in the presence of the PI3K inhibitor it inhibited it by $\sim 2.0\ mg \cdot kg^{-1} \cdot min^{-1}$. The authors thus concluded that impairment of insulin action in the hypothalamus impaired the ability of circulating insulin to inhibit hepatic glucose output. There are several problems with this study, however. First, there were no control experiments in which the rise in systemic insulin did not occur. Secondly, to the extent that either IR-AS treatment or PI3K inhibition impaired the action of basal insulin glucose production, one would have expected a rise in glucose production by the sixth hour of the study. Thus the diminution of the inhibitory effect of the rise in systemic insulin may well be overestimated. Finally, the inhibition of PI3K signalling was not specific to insulin action.

In another study, Pocai et al. [14] investigated the effects of hepatic vagotomy on the ability of a physiological rise in plasma insulin to inhibit hepatic glucose production. In the presence of the vagus nerves, the rise in insulin inhibited glucose production by 78%, while in their absence the same rise in insulin decreased glucose production by only 40%. In other experiments, they showed that this effect reflected an impairment of efferent rather than afferent vagal signalling. While these results support the concept that a portion of insulin's inhibitory action on the liver in the rodent is mediated by the brain, they again are not definitive. It is conceivable that the chronic removal of vagal input had a primary effect on the liver and thereby changed hepatic insulin-sensitivity itself.

Figure 3 | Effects of hepaticdenervation in two groups of overnight-fasted dogs

One group of animals (DN) underwent surgical denervation of the liver approx. 3 weeks earlier. The other group underwent a sham operation. After the control period, insulin was increased by ~30 μ-units/ml in both groups for 3 h (Pe Insulin; bar). The rise in insulin caused net hepatic glucose output to shift to uptake in both groups. Hepatic denervation had no effect on the response of the liver to insulin. Data taken from [16].



In order to determine the role of brain insulin sensing in a non-rodent model, we began a series of studies to address this question in the dog. In order to test the hypothesis that a rise in brain insulin would inhibit hepatic glucose output, we infused insulin at the same rate (0.42 μ-unit/kg of body weight per min^{-1}) as did Rossetti and co-workers [13] into the third ventricle of dogs maintained on a pancreatic clamp. We failed to observe any inhibition of hepatic glucose output [15]. Likewise, when we severed all nerves to the liver and brought about a selective physiological increase in insulin (glucagon basal), the inhibition of glucose output was no different in the sham denervated and the hepatic denervated animals (Figure 3) [16]. The same was true in humans having undergone liver transplantation [17]. Thus, while there is little doubt that the brain of the human and the dog can sense the plasma insulin level [18,19], it does not appear as though that information plays a role in the acute regulation of glucose production by the liver.

Summary

Insulin is a potent inhibitor of hepatic glucose production. Its primary effect on the liver comes about by virtue of its interaction with its hepatic receptors. In addition, however, it can reduce liver glucose output by indirect actions to inhibit lipolysis in the fat cell and to inhibit glucagon secretion from the pancreatic α-cell. These effects occur

across numerous species. The ability of insulin to decrease liver glucose production indirectly by an action on the brain is more controversial. While the brain can certainly sense the circulating insulin level, it is unclear what the physiological consequences of this readout are. In rodents, it appears to play a role in regulating glucose production, but the effect is both slow to be seen and modest in magnitude. No such effect has been demonstrated in the dog or in human.

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