Section 3: Phasic Insulin Release and Metabolic Control

Physiological Consequences of Phasic Insulin Release in the Normal Animal

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The dose-response relationship between the hepatic sinusoidal insulin level and glucose production by the liver is such that a half-maximally effective concentration is at or slightly below the hormone levels seen basally after an overnight fast. In the normal individual, the direct effect of the hormone on the hepatocyte is far more important in restraining glucose production than its indirect effect mediated via a suppression of lipolysis. Because insulin regulates the liver in a direct fashion, its effect occurs within several minutes. Thus, the speed with which insulin works and the sensitivity of the liver to it predict that first-phase insulin release should have a significant effect in quickly suppressing hepatic glucose production. On the other hand, non-hepatic tissues are much less sensitive to insulin and respond slowly as a result of the need for insulin to cross the endothelial barrier. As a result, first-phase insulin is unlikely to significantly alter peripheral glucose disposal. Simulation studies in humans and dogs in which the effects of first-phase insulin were simulated confirmed the aforementioned predictions. In addition, they confirmed the ability of second-phase insulin release to have significant effects on both glucose production and utilization. Diabetes 51 (Suppl. 1):S103–S108, 2002

Insulin plays a key role in glucose homeostasis by virtue of its actions on liver, muscle, and fat. In the liver, it has a sensitive inhibitory effect on glucose production. If one examines data from experiments in the human (1) and the dog (2), it is evident that the half-maximally effective liver sinusoidal insulin concentration is slightly below the insulin level evident within the sinusoids after an overnight fast (Fig. 1). Likewise, it is clear that a threefold increase in insulin above the basal level can almost completely inhibit hepatic glucose release. Thus, the dose-response curve relating insulin to glucose production by the liver is such that small changes in the plasma insulin level up or down can have marked effects on hepatic glucose output. This would predict that first-phase insulin release should have a significant impact on glucose production by the liver.

The question of whether insulin inhibits glucose produc-

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Accepted for publication 12 June 2001.

FFA, free fatty acid.

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The symposium and the publication of this article have been made possible by an unrestricted educational grant from Servier, Paris.

FIG. 1. A: Relationship between the liver sinusoidal insulin level and net hepatic glucose output (NHGO) in overnight fasted conscious dogs maintained on a pancreatic clamp. The sinusoidal insulin level existing in blood as it enters the sinusoids was calculated knowing the arterial and portal vein insulin levels and hepatic arterial and portal vein blood flows. Euglycemia existed for all but the 0 insulin point. That value represents NHGO at 15 min after insulin deficiency when hyperglycemia was mild (~25 mg/dl). Glucagon was clamped at a basal value in all studies. A is reprinted with permission from Cherrington (2). B: The relationship between the estimated hepatic sinusoidal insulin level and tracer-determined glucose production in overnight fasted humans. Euglycemia was maintained by glucose infusion, but glucagon was not clamped. The hepatic sinusoidal insulin level was calculated assuming that portal insulin was three times the arterial insulin and that portal flow contributes 80% of total liver blood flow. B is redrawn from Holther-Nielsen et al. (1).
tion by the liver through direct or indirect means has been the subject of much attention over the past decade (3). It is now clear that the hormone can bring about its effect on the liver through both direct and indirect actions (3). It is also clear, however, that in the normal state, its direct effects are dominant. Figure 2 displays data taken from experiments conducted using the conscious dog in which the arterial insulin level was kept constant at a basal value while the portal (and thus liver sinusoidal) insulin level was changed selectively. To further control the experimental conditions, the plasma glucagon level was kept basal and fixed, and euglycemia was maintained by glucose infusion as required. Hyperglycemia occurred when portal insulin was made deficient, but because the peak glucose production rate was observed after only 15 min of insulin deficiency, the elevation in plasma glucose at that time (≈110 mg/dl) was minimal. If anything, this would have caused an underestimate of the rate of glucose production when portal insulin was deficient. Because the effect of liver sinusoidal insulin on hepatic glucose output reflected glycogenolysis and not gluconeogenesis (the latter did not change), Fig. 2 depicts the net glycogenolytic rate. Clearly, the liver responded to the changes in liver sinusoidal insulin in a sensitive fashion. There is therefore no doubt that insulin reaching the liver is a primary determinant of the extent to which the organ produces glucose. The fact that the plasma free fatty acid (FFA) levels did not change (Fig. 2, bottom panel) provides further evidence that the effects of insulin were direct because the indirect effects of insulin on hepatic glucose production are thought to be mediated by inhibition of lipolysis. In addition, these studies demonstrated that the action of insulin on the liver was rapid in onset, occurring within several minutes.

It is also clear, however, that the effects of insulin on peripheral tissues will modify glucose production indirectly, as suggested by Prager et al. (4), Ader and Bergman (5), and Giacca et al. (6). To the extent that insulin inhibits lipolysis by fat and reduces the plasma FFA levels, it will reduce hepatic glucose output (2,7,8). This reflects the fact that lipolytic inhibition results in decreased FFA uptake by the liver. This in turn results in decreased gluconeogenesis and intrahepatic redirection of glycogenolytically derived carbon such that glucose-6-phosphate undergoes glycolysis with release as lactate rather than dephosphorylation and release as glucose (8). It is clear that in the normal individual the direct effects of insulin on the liver are more important than its indirect effects and explain the majority of the organ’s response to the hormone (3).

The effect of insulin on glucose utilization by muscle and fat has been well characterized (Figs. 3 and 4). The half-maximally effective plasma insulin level is 80–100 μU/ml, a concentration considerably above that needed even for maximal suppression of liver glucose output (9,10). In addition, it is clear that the capacity of muscle to respond to insulin is much greater than the capacity of the liver. Maximally, the liver can decrease glucose production...
by 2.5 mg·kg⁻¹·min⁻¹, whereas muscle can increase glucose uptake by ~8 mg·kg⁻¹·min⁻¹ in humans and almost 14 mg·kg⁻¹·min⁻¹ in the dog. The dog exhibits a more profound increase in insulin-induced glucose uptake because a greater proportion of its muscle mass is comprised of red muscle (high-oxidative fibers). Although the liver can take up glucose under hyperinsulinemic conditions when the blood glucose level is high, it does so minimally under euglycemic-hyperinsulinemic conditions. Thus, under euglycemic conditions, the insulin-induced increase in glucose utilization almost exclusively reflects the action of the hormone on muscle.

The temporal relationship between a change in plasma insulin and the alteration it produces in glucose utilization by muscle and the change it produces in glucose production by the liver is also different. Ader and Bergman (11) have shown that glucose utilization is more closely correlated with the interstitial insulin level than the arterial plasma insulin concentration. Because it takes a finite amount of time for insulin to cross the endothelial barrier, the effect of insulin on muscle glucose uptake is delayed relative to its effect on liver. Insulin gains rapid access to the hepatocytes through the liver sinusoids, which are very permeable to the hormone.

It has been well documented that the release of insulin in response to numerous stimuli, most notably glucose, is biphasic in nature. Early-phase insulin release has two components: a cephalic phase and a first phase. The former is seen with meal consumption and represents the consequence of oropharyngeal stimulation, which causes a rapid (within 2 min) but small (~5 μU/ml) increase in arterial plasma insulin (12,13). So-called first-phase insulin release occurs more slowly (5–10 min) but is much greater in magnitude than the cephalic phase, the extent being directly related to the magnitude of the stimulus in question (14). This early insulin release is believed to represent a pool of insulin stored within the β-cells that can be rapidly mobilized (15). The potential metabolic significance of first-phase insulin release can be appreciated by considering the sensitivity of the liver to insulin and the speed with which it responds. By providing a rapid pulse of insulin, glucose production by the liver can quickly be inhibited, thereby facilitating the response to the metabolic challenge at hand. First-phase insulin release can serve an important function in muscle and fat, but only when it is large and when its contribution to the overall increase in insulin is of the order of ≥50% (16). Getty et al. (16) have shown that a large first-phase insulin release allows the interstitial insulin level to rise more rapidly than would otherwise be the case. Therefore, even though insulin must first cross the endothelial barrier to act, muscle can be called upon more quickly to deal with the metabolic challenge at hand. In the study by Getty et al., first-phase insulin release augmented glucose utilization between the 10- and 30-min time points.

Glucagon is a potent regulator of glucose production by the liver. In an earlier study by Steiner et al. (17), a rise in glucagon resulting from intraportal infusion of the hormone caused a rapid (7.5 min) but limited (~1.8 mg·kg⁻¹·min⁻¹) increase in glucose production and a delayed but quantitatively similar rise in glucose utilization. The hyperglycemia that resulted (~20 mg/dl) was thus minimal because the two rates of glucose flux were unequal for just a brief period. The key to the prevention of excessive hyperglycemia was the biphasic response of insulin secretion. The latter rose from 7 ± 2 to 34 ± 3 μU/ml in 5 min and then fell to a value of 21 ± 31 μU/ml at 30 min and eventually plateaued at 14 ± 2 μU/ml. Steiner et al. (17) examined the importance of first- and second-phase insulin release to glucose homeostasis in the face of this glucagon challenge using the pancreatic-clamped conscious dog. In the absence of any insulin release (basal plasma insulin was maintained by portal insulin infusion in the presence of somatostatin), a rise in glucagon from 82 ± 9 to 293 ± 50 pg/ml increased glucose production from 2.9 ± 0.2 to 10.1 ± 1.1 mg·kg⁻¹·min⁻¹ within 7.5 min. At the same time, glucose utilization rose from 2.9 ± 0.3 to 3.8 ± 0.8 mg·kg⁻¹·min⁻¹. Glucose production exceeded glucose utilization for ~90 min, thus resulting in hyperglycemia of 223 ± 16 mg/dl. In the last hour of the 3-h test period, glucose production averaged 3.9 ± 0.4 mg·kg⁻¹·min⁻¹, whereas glucose utilization averaged 4.1 ± 0.3 mg·kg⁻¹·min⁻¹, and the plasma glucose level drifted down slowly.

When the experiment was repeated but first-phase insulin alone was simulated (7 ± 2 to a peak of 25 ± 4 μU/ml at 5 min and back to 7 ± 2 μU/ml by 30 min), the results were markedly different. Glucose production rose by only 1.5 ± 0.5 mg·kg⁻¹·min⁻¹ at 7.5 min. Once again, glucose utilization changed minimally (Δ0.4 ± 0.3 mg·kg⁻¹·min⁻¹). As a result of the blunted increase in glucose production, the glucose level did not increase significantly in the first 15 min. Over the first hour of the study, the first-phase insulin response reduced the increase in glucose production by ~70%, but it had no significant effect on glucose utilization. By the last hour of the study, there was no evidence of any continuing effect of the early pulse of insulin on glucose turnover. In fact, the rates of glucose production and utilization resembled those seen in the group in which insulin was kept basal.

In the next experimental protocol, second-phase insulin release was simulated. The rise in glucagon was similar to
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response ( was augmented by second-phase insulin release. rise in plasma glucose, it is clear that glucose clearance
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result, the plasma glucose level rose by a maximum of 40 kg
1, although by 15 min, it had risen by 1.8 mg · kg−1 · min−1. Glucose utilization rose more rapidly than in the previous two protocols. As a result, the plasma glucose level rose by a maximum of 40 mg/dl (30 min), after which it drifted back toward baseline. Because glucose utilization rose in the absence of a large rise in plasma glucose, it is clear that glucose clearance was augmented by second-phase insulin release.

In the presence of complete simulation of the insulin response (first and second phase), the glucose production and utilization responses were normalized, and a rise in plasma glucose of only ~20 mg/dl occurred. The early (7.5-min) rise in glucose production was 1.0 mg · kg−1 · min−1, although by 15 min, it had risen by 1.8 mg · kg−1 · min−1. Glucose utilization rose quite rapidly and, by 45 min, equaled glucose production. Once again, because glucose utilization rose in the presence of a minimally altered plasma glucose level, glucose clearance rose significantly. Thus, the overall response to glucagon seen in the normal animal was restored.

The effects of first-phase insulin release can be assessed in two ways: by looking at its ability to improve the poor glucose tolerance seen in the presence of basal insulin or by examining its effect in the presence of first-phase insulin release (Fig. 4). In the former case, it reduced the overall increase in plasma glucose by 31%. In the presence of second-phase insulin secretion, first-phase insulin release improved the plasma glucose profile by almost 50%. In each case, the quick rise in insulin produced the majority of its effect by blunting the glucagon-induced early rise in glucose production (Fig. 5). The early rise in insulin had no significant effect on glucose clearance, but it should be noted that the change in area under the curve due to first-phase insulin was only 20% of the overall change in area under the curve for the hormone. Thus, reduced glucose production in the first 15–30 min was the key to improving glycemia. The reason that first-phase insulin release produced a prolonged improvement in glycemia is that it prevented the early accumulation of glucose in the vascular space, which occurs when the rate of glucose appearance is greater than the rate of glucose disappearance. It should be remembered that if first-phase insulin were to be selectively defective in an otherwise normal individual, the increased initial glycemia would lead to enhanced second-phase insulin release. This would eventually reestablish a near-normal glucose level. To the extent that second-phase insulin release cannot compensate for a defective first-phase release, excessive hyperglycemia would be sustained.

The effects of second-phase insulin release can also be estimated in two ways: by looking at its ability to improve glycemia in the presence of basal insulin or by examining its effect in the presence of first-phase insulin release. In the former case, it reduced the overall increase in plasma glucose by almost 76%. In the presence of first-phase insulin secretion, second-phase insulin release improved the plasma glucose profile by almost 83%. In the former case, the improvement was a function of a rapid and prolonged decrease in glucose production as well as an improvement in glucose clearance, which became evident by 75 min (Fig. 6). It should be remembered that the second-phase insulin release began coincident with the glucagon stimulus rather than in the delayed fashion, thus explaining the quick (even though limited) action of the hormone on the liver. When second-phase insulin release was added to the first-phase secretion, there was a modest decrease in glucose production and a significant increase in glucose clearance. Thus, second-phase insulin secretion brings about its impact on glycemia because of its effect on both glucose utilization and production.

In summary, first-phase insulin release serves to rapidly inhibit glucose production. This result is consistent with its potent and rapid action on the liver. On the one hand,
first-phase insulin release has little effect on glucose uptake by muscle. This is also consistent with the less potent and less rapid action of insulin on muscle. On the other hand, second-phase insulin release predictably has potent and less rapid action of insulin on muscle. This is also consistent with the less rapid rise in glucose clearance, on the other hand, second-phase insulin release to the suppression of glucose production by the liver while having no discernable effect on glucose utilization or clearance.

Based on the above, one would predict that in a more physiological circumstance when plasma glucose rises (i.e., food or glucose intake), the quick first-phase release of insulin would cause the plasma level of insulin to rise more rapidly than it otherwise would, even if the early burst of insulin secretion does not result in a discrete spike in plasma. The question thus arises as to the significance of such priming. In addition, studies to date have only addressed the significance of first-phase insulin release to the suppression of glucose production by the liver and not to the ability of the liver to take up and store glucose. The question thus arises as to whether first-phase insulin release plays a significant role in causing glucose uptake by the liver either initially (coincident with the elevation in plasma insulin) or later in response to the meal.

In that regard, we carried out a study (19) in which we created hyperglycemia (~220 mg/dl) in the conscious dog in the presence of basal insulin and glucagon levels (maintained with a pancreatic clamp). After 2 h of selective hyperglycemia, which suppressed net hepatic glucose output to zero, the insulin level was increased fourfold or left at a basal value. A square-wave rise in liver sinusoidal somatostatin, it is possible to approximate the portal insulin levels present in their two protocols over the first 10 min of the response to glucose. In the group in which first-phase insulin was present, it would appear that portal insulin was ~50 μU/ml. In the absence of first-phase insulin release, portal insulin can be estimated to be ~30 μU/ml. Given that basal portal insulin was ~20 μU/ml, one would predict from the dose-response data shown earlier that a significantly greater initial fall in glucose production would occur when first-phase insulin release was simulated. These data thus agree with those of Steiner et al. (17), which showed that first-phase insulin release serves to rapidly inhibit glucose production by the liver while having no discernable effect on glucose utilization or clearance.

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insulin from 22 ± 4 to 67 ± 8 μU/ml had little (~0.3 mg · kg⁻¹ · min⁻¹) effect on net hepatic glucose uptake for 30 min. In fact, it took 60 min for net hepatic glucose uptake to become significant. It was thus concluded that, although insulin can rapidly shut down hepatic glucose production, it cannot, alone at least, quickly cause the liver to take up glucose significantly. In contrast to the action of insulin, the effect of portal glucose delivery (the “portal” signal) on net hepatic glucose uptake was rapid, reaching a peak net hepatic glucose uptake (2.4 mg · kg⁻¹ · min⁻¹) within 15 min. Perhaps in the presence of the portal signal, insulin could compensate for the lack of insulin release could compensate for the lack of insulin sensitivity in the early phase of a response to a glucose load or that it would work in the presence of orally derived glucose. It also remains unclear whether an early spike of insulin could have a long-term benefit on the liver’s ability to take up and store glucose. We are currently examining both of these possibilities.

ACKNOWLEDGMENTS
This research was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-18243, DK43706, and 5P60 DK20593 (Diabetes Research Training Center).

REFERENCES