The Hepatic Glucose Response to Insulin in the Unanesthetized Dog*

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The precise action of insulin on the liver remains open to investigation and understanding despite considerable data which have been accumulated. Dunn et al. (1) with the use of C14-labeled glucose in the dog, express one point of view when they state: "These isotope tracer studies, which clearly indicate an action of insulin in inhibiting glucose output, may be added to a growing body of information which is making it increasingly obvious that a major action of insulin is exerted on the liver." These writers add that their observations "... extend these earlier findings by demonstrating that insulin does in fact act immediately to inhibit glucose production by liver."

In liver slices of diabetic rats glucose uptake is subnormal and glucose output is excessive. As measured in this system, Renold et al. (2) found that the diabetic lesion is not immediately corrected by the addition of insulin, but requires several hours to several days. Haft and Miller (3) perfusing the isolated rat liver with a medium containing 350 mg. of glucose per 100 ml., found this value rising to 600 mg. in 1 hour, unaffected by insulin. In the alloxan diabetic rat liver insulin produced an increased removal of glucose from the medium between 1.5 and 4.0 hours of perfusion at these glucose concentrations (3). No such effect has been demonstrable in the cat or rabbit liver (4) even though fatty acid synthesis (5), peptide synthesis (6), and incorporation of C14 labeled glucose into glycogen (7) can be stimulated by insulin administration (Bondy et al. (10)). This observation has been confirmed by Sherlock et al. (11) with the conclusion that insulin produces an hepatic as well as peripheral uptake of glucose. On the other hand, measurement of portal-hepatic venous glucose concentrations shows no change in the hepatic glucose gradient after insulin in given to normal unanesthetized dogs (Ashmore et al. (12)). The present study is undertaken to provide more direct quantitation of insulin-liver phenomena, our results indicating that insulin has no direct immediate effect upon the liver of the dog.

EXPERIMENTAL

Two techniques which have been devised in this laboratory are used in this study. The first consists of direct perfusion of the dog liver in vivo (13), and the second utilizes simultaneous catheterization of the hepatic vessels in a manner previously described (14). These methods are invoked in an attempt to illuminate that area between the liver slice and the intact organism. In the perfusion technique blood flow and pressure are directly controlled with the aim of maintenance of viability together with minimal alteration of the normal equilibria of the hepatic cell. The second maneuver permits repeated observations of the catheterized system in an unanesthetized and otherwise intact animal.

Direct Perfusion Studies

Seven perfusion experiments were performed. After light morphine Thiopental anesthesia, cannulas were placed in the portal vein and vena cava. A reservoir of oxygenated, constant temperature blood from a donor dog was connected to cannulas by means of a pump oxygenator. Simultaneously the hepatic and systemic circulations were completely separated. At no time was there cessation of oxygenated blood flow to the liver under normal minute volume and pressure. These factors, plus avoidance of hepatic manipulation, were thought to be responsible for the lack of hepatic swelling and engorgement which have been observed by Tröwell (15), Andrews et al. (16), Lundsgaard (4), and Bauer et al. (17). An average of 540 ml. of blood obtained from donor dogs was used to prime and maintain the extracorporeal circuit. This, together with an estimated 180 ml. in the liver and its large vessels constituted the volume of the perfusate. When the circuit was established, only blood samples were withdrawn and none was added.

Equilibration and control studies occupied the initial 15 to 30 minutes of perfusion, including sampling of the perfusate and hepatic biopsy. Glucagon-free insulin (Lilly), 2 units per kg., was administered via the portal venous (inflow) catheter, and further sampling and biopsy continued at intervals during the ensuing hour. Glucose of the blood perfusate was determined by the method of Nelson-Somogyi (18) and liver biopsy glycogen determined by the method of Roe (19).
Ten experiments were performed in 9 dogs. As a preliminary step, the animals had portal, hepatic, and splenic arterial catheters placed under Thiopental anesthesia, standard aseptic technique being used. When the animals had completely recovered, 2 to 8 days later, studies were carried out in the unanesthetized, nonsedated, postprandial state. Simultaneous blood samples were taken from each catheter at 5 to 15 minute intervals before and after insulin with replacement of blood from donor dogs in most instances.

Measurement of hepatic blood flow was determined by a modified Bromsulphalein method previously described (20).

Simultaneous measurements of portal, hepatic venous, and arterial concentrations together with knowledge of hepatic blood flow, permit a quantitative assessment of metabolic events in various anatomic regions. For our purpose the hepatic venous-arterial difference represents the entire splanchnic gradient; the portal venous-arterial difference represents the nonhepatic-splanchnic gradient; while the hepatic venous-portal difference renders the hepatic gradient. The hepatic gradient thus considered approximates the total hepatic gradient under baseline conditions since 80 per cent of the hepatic inflow in the unanesthetized dog is by way of the portal vein (21). With this ratio of portal venous to hepatic arterial inflow, the magnitude of error for calculations from this system is 6 per cent.

**Table I**

<table>
<thead>
<tr>
<th></th>
<th>Initial, before perfusion</th>
<th>15 min. perfusion, no insulin</th>
<th>60 min. perfusion, 45 min. after insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog 1</td>
<td>208</td>
<td>103</td>
<td>103</td>
</tr>
<tr>
<td>Dog 2</td>
<td>158</td>
<td>109</td>
<td>93</td>
</tr>
<tr>
<td>Dog 3</td>
<td>350</td>
<td>295</td>
<td>370</td>
</tr>
<tr>
<td>Dog 4 (control)</td>
<td>165</td>
<td>89</td>
<td>100†</td>
</tr>
</tbody>
</table>

* Values are given as mmoles per gm. wet liver.
† No insulin added.

Glucagon-free insulin (Lilly) or Novo-Insulin¹ was injected via an indifferent vein or arterial catheter in doses of 0.1 to 0.2 unit per kg. after the control period.

1 Kindly supplied by Professor C. de Duve.
RESULTS

Effect of Insulin on Perfused Liver

Glucose Levels of Perfusate before and after Insulin—In control experiments without insulin, the control glucose concentration of the perfusate was in the range of 55 to 150 mg. per 100 ml. In these perfusion experiments glucose values rose slowly and then tended to plateau at approximately 200 to 300 mg. per 100 ml. by 60 to 90 minutes. The increment in glucose was largely explained by the continued hepatic glucose output, maintained at approximately 2 to 3 mg. per kg. per minute, into the perfusing blood, with no extrahepatic glucose utilization. In those perfusion experiments regarded as technically satisfactory, the increment of glucose was consistent and serum potassium levels were minimally changed. Blood flow was constant at a constant in-flow pressure. In technically less satisfactory experiments where complete hepatic viability was not assumed, there were abrupt increases in glucose and potassium.

Glucose concentrations of the perfusate from the entire series of the perfused livers are summarized in Fig. 1.

Hepatic Glycogen Content of Biopsies—Glycogen analysis of liver biopsies taken before perfusion, during the control period, and after insulin administration showed variability. Such variations were also present in a control perfusion in which no insulin was added. Nevertheless, no significant increase in hepatic glycogen was observed after insulin administration. These data are summarized in Table I.

Experiments in vivo: Catheterization of Vessels

Glucose Concentrations—A fall in the glucose concentrations was observed after insulin (Fig. 2). This is noted at 5 minutes, data), insulin was found to have no direct immediate effect upon the splanchnic and hepatic glucose output. Rather, insulin produces an uptake of glucose, with no extrahepatic glucose utilization. In those experiments regarded as technically satisfactory, the decrease of glucose was consistent and serum potassium levels were minimally changed. Blood flow was constant at a constant in-flow pressure. In technically less satisfactory experiments where complete hepatic viability was not assumed, there were abrupt increases in glucose and potassium.

Glucose concentrations of the portal venous, hepatic venous, and arterial plasma was 47, 41, and 39 mg. per 100 ml., respectively.

Glucose Concentration Gradients—The total splanchnic hepatic and nonhepatic splanchnic glucose concentration gradients in the control period and during the first 30 minutes after insulin are summarized in Table II. There is significant decrease in the total splanchnic and nonhepatic splanchnic glucose gradients, but no decrease in the hepatic gradient.

Splanchnic Glucose Output and Hepatic Glucose Output—Since statistically significant flow alterations were not observed, the concentration gradients reflected their respective regional glucose output rates (Table II). The calculated splanchnic glucose output decreased, whereas concomittantly the hepatic glucose output remained the same or slightly increased in each of the animals studied. Thirty minutes after the insulin administration, maximal hypoglycemia was reached; both increased total splanchnic and hepatic glucose output were then found.

SUMMARY

In an attempt to characterize the movements of glucose in response to insulin, direct simultaneous measurement of concentration gradients across the liver, total splanchnic and nonhepatic splanchnic areas have been combined with a measurement of plasma flow. This allows direct comparison of events in each of these anatomical regions as a function of time after the introduction of an experimental variable. Thus, regional metabolism of the splanchnic system may be assessed directly and the metabolic events occurring in the liver may be differentiated from those occurring in the area drained by the portal vein.

Within this framework (and corroborated by direct perfusion data), insulin was found to have no direct immediate effect upon hepatic glucose output. Rather, insulin produces an uptake of glucose by the nonhepatic splanchnic tissues.

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