DIRECT STIMULATION BY GLUCOSE AND INSULIN OF GLYCOGEN SYNTHESIS IN PERFUSED RAT LIVER

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1. Introduction

The central role of the liver in blood glucose regulation was first demonstrated in the intact animal [1] with inhibition of glucose production under a glucose load. Insulin plays a key role in this regulatory process, diminishing hepatic glucose output and accelerating glycogen deposition [2]. While this situation appears in the intact animal, studies on isolated liver preparations have given inconclusive results. For example, in perfused liver and isolated hepatocytes inhibition of net glucose output and stimulation of glycogen synthesis occurs only at unphysiologically high concentrations of glucose [3–5] or gluconeogenic substrates [3,5] and is insulin-insensitive [6]. A defect in isolated liver preparations has been proposed [2].

Insulin insensitivity of the perfused rat liver depends upon the use of whole blood as perfusate and that the hormone responsiveness was lost following dilution of blood with buffer [7]. It was of interest to determine whether insulin enhanced hepatic glucose uptake and glycogen synthesis in livers perfused with whole blood. Here, we report that there was a substantial insulin-dependent uptake of added glucose by the perfused liver which was, additionally, accelerated by the hormone. Glycogen synthesis paralleled the rate of glucose uptake and was increased by insulin.

2. Materials and methods

Livers from fed adult male rats of the Hooded Wistar strain (295–305 g) were perfused in situ for 60 min. The perfusate was 80 ml defibrinated whole rat blood, dialyzed to remove vasoconstrictive factors [9] and perfused at 11 ml/min. At time zero infusion of glucose and insulin was started. The hormone was added to maintain 100 ng/ml of serum [7] while glucose was infused initially at 200 μmol/min for 1.5 min and subsequently at 20 μmol/min [10]. Both infusions were in 0.15 M NaCl and control livers were infused with 0.15 M NaCl only.

Blood samples were taken at zero time and then at 15 min intervals and assayed for glucose and lactate [7]. At the end of the experiment a portion of liver (1.0–1.3 g) was quickly taken, blotted dry and weighed for glycogen estimation [10]. The remainder of the liver was similarly weighed for calculation of metabolic activity/g liver. Bile was collected at 15 min intervals [7].

All data are shown as the mean ± SEM of the numbers of observations in parentheses. Statistical significance was determined by analysis of variance.

3. Results and discussion

3.1. *Bile flow*

The rate of bile flow did not differ between the control and glucose-infused livers with mean values of 740 ± 30 (4) and 760 ± 50 (5) μl . liver^{-1} . h^{-1}, respectively. Production was significantly ($P < 0.001$) increased to 950 ± 50 (4) μl . liver^{-1} . h^{-1} in the insulin group. Rather surprisingly this stimulation was abolished in the insulin + glucose group with a mean flow of 780 ± 50 (5) μl . liver^{-1} . h^{-1}.

In this system bile flow is largely bile acid-independent and therefore represents ion transport [7]. The increased production most probably reflects altered hepatocellular ionic balance and may mediate some of the metabolic effects of insulin [7,8]. Whether the loss of hormonal stimulation in the glucose + insulin group is a cause or merely an effect of some of the metabolic effects described below remains to be established.

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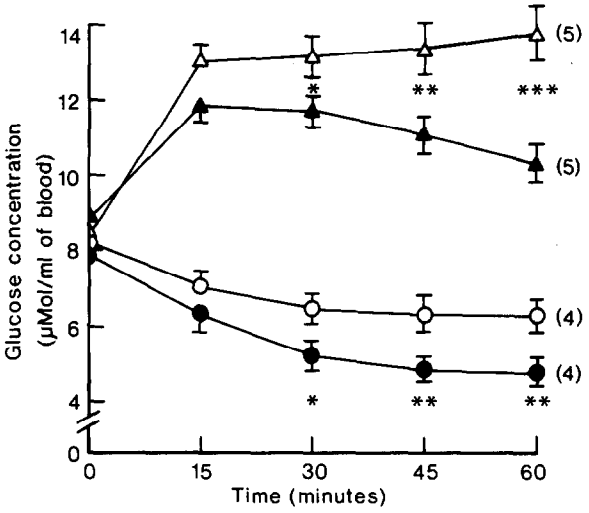

Fig.1. Effects of glucose and insulin on blood glucose concentrations in perfused livers from fed rats: (○) control; (●) insulin: (△) glucose; (▲) glucose + insulin; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ insulin *vs* control or glucose + insulin *vs* glucose, respectively.

3.2. *Insulin and the hepatic glucose metabolism*

Zero-time concentrations of glucose did not differ between the 4 experimental groups with a mean value of 8.4 μmol/ml (fig.1). In control livers, glucose levels stabilized at 6.2 μmol/ml and, as observed in [7,8], the equilibrium concentration was significantly lowered by insulin. Following glucose infusion, concentrations rose sharply at 0–15 min and then more slowly for the rest of the experiment. Although the simultaneous infusion tended to lower perfusate glucose at 15 min, it was 30 min before the effect was significant, statistically. At the end of the experiment mean blood glucose was 3.5 μmol/ml lower in the glucose + insulin group as compared with the glucose group.

Table 1
Effects of insulin and glucose on net glucose uptake and liver glycogen content in perfused rat liver (μmol/liver)

Additions to perfusate	*n*	Glucose uptake	Glycogen content
None (control)	4	179 ± 39	2070 ± 140
Glucose	5	908 ± 48[b]	2530 ± 170[a]
Insulin	4	390 ± 31[a]	2540 ± 140[a]
Glucose + insulin	5	1190 ± 37[b]	3100 ± 130[b]
Non-perfused controls	6	–	1970 ± 130

[a] $P < 0.05$, [b] $P < 0.001$ *vs* perfused control

Calculation of net hepatic glucose uptake for the whole experiment, based on the rate of infusion and change in perfusate concentration [10] showed that in control livers there was a net uptake of ~180 μmol (table 1). Because of the lower equilibrium concentrations in the livers infused with insulin, net uptake was correspondingly larger at 390 μmol/liver. The net removal of glucose was stimulated by the infusion of the hexose with a total removal of 900 μmol. As might be inferred from the time course of glucose concentration, total uptake was significantly enhanced in the livers infused with hexose + insulin and the increase was of the same order of magnitude as between the control and insulin group. The greatest increase in uptake however was in the glucose group.

Liver glycogen content was measured in all groups at the end of perfusion and also in 6 animals which were sampled immediately after the isolation procedure to give an indication of zero time values (table 1). As the mean glycogen content of the latter was the same as perfused controls, it is clear that the increased content of the other groups reflects net glycogen synthesis. Total glycogen was significantly increased by insulin, presumably via cAMP [2], or Ca^{2+}- or K^+-dependent alterations in phosphorylase and/or glycogen

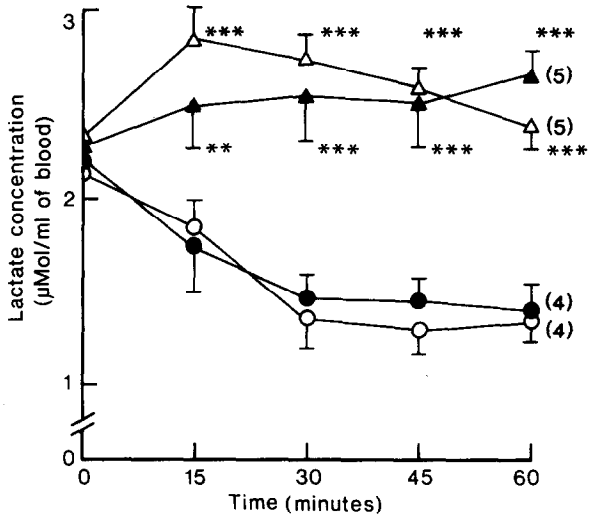

Fig.2. Effects of glucose and insulin on blood lactate concentrations in perfused livers from fed rats: (○) control; (●) insulin; (△) glucose; (▲) glucose + insulin; ** $P < 0.01$, *** $P < 0.001$ *vs* control.

synthase activities [11]. The increase in glycogen in livers infused with glucose was of similar magnitude to that in the insulin group and probably reflected the sequential inactivation of phosphorylase and stimulation of the synthase by the hexose [2]. Accumulation of glycogen was greatest in livers perfused with glucose + insulin and was equivalent to the combined increases in the glucose and insulin livers.

3.3. *Blood lactate*

Because of the apparent necessity for gluconeogenic precursors for net glycogen synthesis in other liver preparations [3,5] blood lactate concentrations were measured. This was chosen as it is the major gluconeogenic substrate present under these conditions [12].

Lactate concentrations reached equilibrium at ~1.4 μmol/ml in both control and insulin-infused livers (fig.2). However, when glucose was infused (alone or with insulin) equilibrium was reached at significantly higher concentrations of 2.4–2.6 μmol/ml. These data show that there was no consistent relationship between perfusate lactate and liver glycogen. The results also differ considerably from experiments where glucose was added at 50% of the present rate and in which lactate concentrations were unaltered [10]. The present rate of addition was selected to approximate to the rate of entry of glucose into the hepatic portal vein from the intestine under saturating conditions [13] and the elevation in blood lactate is similar to that observed when fructose is metabolised by the perfused liver [10]. In liver the latter monosaccharide is regarded as being under a lesser degree of metabolic control than glucose [14] and raises the concentrations of glycolytic intermediates [15]. It may well be that at the present rate of uptake, glucose was also able to overcome some of the metabolic controls exerted on glycolysis.

4. Conclusions

From these studies it appears that the isolated liver perfused with whole blood, prepared as in [9], closely resembles the organ in the intact animal. Thus, there is a very substantial uptake of glucose in the absence of raised insulin levels while uptake is enhanced by addition of the hormone [16]. In contrast to most other in vivo systems there is no apparent defect in the insulin responsiveness of glucose uptake and glycogen synthesis. Further, there is no consistent relationship between the latter process and the concentrations of gluconeogenic precursors (in this case, lactate). The present observations may reflect the choice of perfusate with its excellent capacity to supply O_2 under physiological conditions [7,8].

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