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Hypoglycemia induced by insulin increases hepatic capacity to produce glucose from gluconeogenic amino acids[1]

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KEY WORDS hypoglycemia; insulin; gluconeogenesis; liver glycogen; hepatic metabolism

ABSTRACT

AIM: To investigate the hepatic capacity to produce glucose during hypoglycemia induced by insulin (HII). **METHODS**: Livers from 24-h fasted rats which received ip insulin (HII rats) or saline (control rats) were perfused *in situ*. The gluconeogenic substrates *L*-alanine (5 mmol/L), *L*-glutamine (5 mmol/L), *L*-lactate (2 mmol/L), and glycerol (2 mmol/L) were employed. The gluconeogenic activity was measured as the difference between rates of glucose released during and before the substrate infusion. In part of the experiments the production of urea was measured. Before the liver perfusion blood was collected for determination of glycemia and insulinemia. **RESULTS**: HII rats showed: (a) hypoglycemia and hyperinsulinemia; (b) increased hepatic capacity to produce glucose from *L*-alanine and *L*-glutamine; (c) increased hepatic ureogenesis from *L*-alanine and *L*-glutamine; and (d) increased hepatic glucose production from glycerol. However, hepatic glucose production from *L*-lactate was not affected by hypoglycemia. **CONCLUSION**: In spite of hyperinsulinemia the hepatic capacity to produce glucose from *L*-glutamine and *L*-alanine increased during HII. These results can be attributed to the higher hepatic catabolism of both amino acids, since the ability of the liver to produce glucose was not affected by hypoglycemia.

INTRODUCTION

The ability of the liver to produce glucose is crucial to the maintenance of a supply of glucose for the brain. This is particularly critical during hypoglycemia induced by insulin (HII), a condition where the consumption of glucose is markedly increased.

In addition, it is well established that insulin inhibits the mobilization of amino acids and key enzymes of gluconeogenesis[1], but it must be considered that HII stimulates the release of anti-insulin hormones[2,3], which increase the rate of hepatic gluconeogenesis[3,4]. Thus, the maximal capacity of the liver to convert amino acids to glucose during HII is not clear, since the plasma levels of amino acids increase[5] or decrease[6,7] depending on the experimental conditions. In addition, the liver is simultaneously submitted to the influence of insulin injected and endogenous anti-insulin factors. To clarify this question, we employed isolated liver, which received saturating concentration of gluconeogenic substrates. This experimental approach discards the influence of changing in the flux of amino acids to the liver.

Since gluconeogenesis is vital to glucose recovery, if the hepatic glucose production from amino acids, during HII, is maintained, these findings might have significant implications. Therefore, the aim of this investigation was to determine whether the maximal capacity of the liver to produce glucose from *L*-alanine and *L*-glutamine was affected by HII.

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MATERIALS AND METHODS

Rats Albino ♂ Wistar rats weighing about 200 g were starved (24 h) and divided in two groups. The HII group was composed by rats, which received an ip injection of regular insulin ($1.0\ U \cdot kg^{-1}$). Rats that received an ip injection of saline composed the control group.

Experimental design Because hypoglycemia was well established 1 h after insulin administration[8], this time was selected to start the liver perfusion. Thus, at this time, the rats were anesthetized by ip injection of pentobarbital ($40\ mg \cdot kg^{-1}$) and after laparotomy, blood was collected from cava vein for determination of glycemia[9] and insulinemia[10]. The livers were perfused *in situ* as described previously[11,12].

After a pre-perfusion period, precursors of hepatic glucose (*L*-alanine, *L*-glutamine, *L*-lactate, and glycerol) were infused and the gluconeogenic activity was measured as the difference between rates of glucose released during and before the substrate infusion. Samples of the effluent perfusion fluid were collected in 2-min intervals and analyzed for *D*-glucose[9]. In addition, hepatic urea production from *L*-alanine and *L*-glutamine was measured[13].

Reagents *L*-alanine, *L*-glutamine, and *L*-lactate were purchased from Sigma, USA. All other reagents were of the highest purity obtainable.

Statistical analysis Statistical analyses were performed using *t* test or ANOVA using the program Primer biostatistics (version 1.0). The results in the text are presented as $\bar{x} \pm s$. A 95 % level of confidence ($P < 0.05$) was accepted for all comparisons.

RESULTS

One hour after insulin administration, plasma insulin levels increased ($P < 0.05$) from (27.0 ± 5.3) $mU \cdot L^{-1}$ to (136.3 ± 26.4) $mU \cdot L^{-1}$ and glycemia decreased ($P < 0.05$) from (4.9 ± 0.10) mmol/L to (2.5 ± 0.06) mmol/L.

Livers from hypoglycemic rats showed increased ($P < 0.05$) hepatic capacity to produce glucose from *L*-alanine (Fig 1A) and *L*-glutamine (Fig 1B). Similar results were observed for ureogenesis, since urea production from *L*-alanine (Fig 1C) and *L*-glutamine (Fig 1D) were increased ($P < 0.05$).

In addition, glucose production from *L*-lactate was not affected by hypoglycemia (Fig 2A). However, hepatic glucose production from glycerol was stimulated ($P < 0.05$) by hypoglycemia (Fig 2B).

DISCUSSION

Although the levels of blood glucose precursors like amino acids, glycerol, and *L*-lactate changed[2-7] during hypoglycemia, the capacity of the liver to produce glucose from these substrates during glucose recovery is not clear. To clarify this question we employed isolated liver from fasted rats, which received saturating concentration of gluconeogenic substrates. This experimental approach discards the influence of hepatic glycogen catabolism and the variability of hepatic glucose precursors to the liver[11,12].

Thus, the capacity of the liver to produce glucose from gluconeogenic precursors during HII was investigated.

Firstly, the production of glucose from *L*-alanine and *L*-glutamine, quantitatively important amino acids precursors of glucose in the liver, were investigated. In spite of hyperinsulinemia, livers from hypoglycemic rats showed increased ($P < 0.05$) glucose production from *L*-alanine (Fig 1A) and *L*-glutamine (Fig 1B). The increased hepatic glucose production from both amino acids can be attributed to the fact that the catabolism of *L*-alanine and *L*-glutamine was increased during HII. In agreement with this suggestion, the urea production from *L*-alanine (Fig 1C) and *L*-glutamine (Fig 1D) was increased ($P < 0.05$) in livers from hypoglycemic rats.

Since hypoglycemia also increased the ability of the liver to produce glucose from pyruvate (not showed) and glycerol (Fig 2B), substrates which enters the gluconeogenic pathway respectively at the first and triose phosphate step. The absence of an increased glucose production from *L*-lactate (Fig 2A) in livers from HII rats was an unexpected result. In agreement with this observation, Mokuda and Sakamoto[3] demonstrated an increased incorporation of ^{14}C from *L*-lactate into blood glucose, 5 min after an iv injection of insulin, ie before the release of counter-regulatory hormones. One possibility, to explain our results for *L*-lactate, is that HII rats, like diabetic rats[14], showed increased cytosolic $NADH/NAD^+$

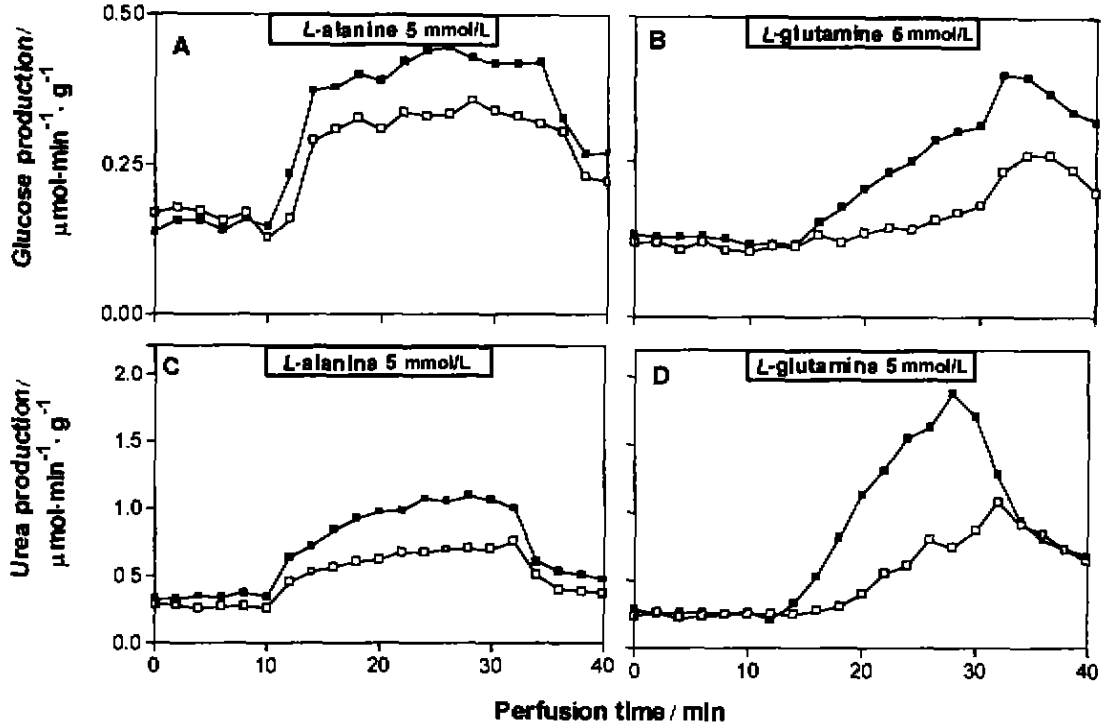

Fig 1. Glucose and urea production from *L*-alanine (left side) and *L*-glutamine (right side) in perfused liver from saline (□) and insulin injected rats (■). The effluent perfusate was sampled in 2-min intervals and analyzed for glucose and urea. Values are means of 5 experiments. The area under curves for saline (normoglycemic) and insulin (hypoglycemic) injected rats were: A (4.9 ± 0.7) μmol·g^{-1} *vs* (7.6 ± 1.1) μmol·g^{-1}, B (2.2 ± 0.2) μmol·g^{-1} *vs* (4.4 ± 0.8) μmol·g^{-1}, C (9.9 ± 0.6) μmol·g^{-1} *vs* (15.4 ± 1.3) μmol·g^{-1}, and D (12.5 ± 2.6) μmol·g^{-1} *vs* (24.4 ± 3.6) μmol·g^{-1}, respectively.

ratio, which decreased the ability of the liver to convert *L*-lactate to pyruvate, a crucial step to start gluconeogenesis from *L*-lactate.

In summary, pharmacological doses of insulin increase the hepatic capacity to produce glucose. Thus, we can conclude that the counterregulatory hormones released during HII[2-7] overcome the insulin inhibition on key enzymes of gluconeogenesis and the hepatic ability to produce glucose not only was maintained (*L*-lactate), but also stimulated (for *L*-alanine, *L*-glutamine, glycerol).

These findings give the possibility for using amino acids supplementation to treat HII. In fact, this possibility was investigated previously[6,7], but further investigations are necessary to establish the importance of amino acids administration to glucose recovery.

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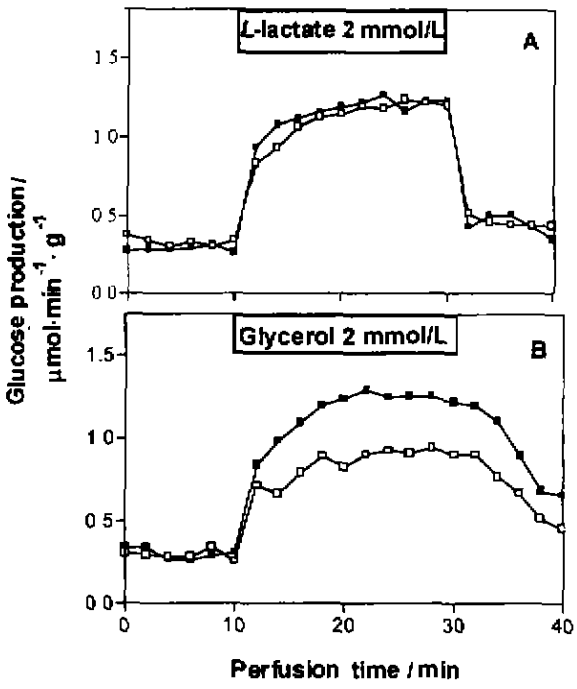

Fig 2. Glucose production from *L*-lactate (above) and glycerol (down) in perfused liver from saline (□) and insulin injected rats (■). The effluent perfusate was sampled in 2-min intervals and analyzed for glucose. Values are means of 5 experiments. The area under curves for saline (normoglycemic) and insulin (hypoglycemic) injected rats were: A (16.3 ± 1.8) μmol·g^{-1} *vs* (19.4 ± 2.1) μmol·g^{-1}, and B (15.7 ± 1.8) μmol·g^{-1} *vs* (23.4 ± 1.6) μmol·g^{-1}, respectively.

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胰岛素引起的低血糖增加肝脏通过糖原异生氨基酸产生葡萄糖的能力[1]

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关键词 低血糖症; 胰岛素; 糖原异生; 肝糖原; 肝脏代谢

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