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Concentration Dependent Inhibition of Hepatic Gluconeogenesis by Insulin (38068)

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(Introduced by J. H. Annegers)

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Insulin in its role as a hypoglycemic agent, stimulates glucose uptake and utilization by muscle and adipose tissue. While insulin has been shown to decrease the net release of glucose by the liver (1–6), the degree to which changes in gluconeogenesis contribute to this response is uncertain. Insulin has been shown to inhibit hepatic glucose production from lactate (7) and alanine (8), however, both the substrate and insulin concentrations employed in these studies were well above those occurring under physiological conditions. In order to investigate the importance of inhibition of hepatic gluconeogenesis as a mechanism involved in the hypoglycemic action of insulin, it is necessary to examine this action at physiologically occurring substrate and insulin concentrations.

Since insulin is secreted directly into the portal circulation and is rapidly degraded by the liver, normal portal venous insulin concentrations are higher than peripheral levels. In man, mean values for portal venous insulin range from 37 μU/ml in the fasting state to 500 μU/ml following an intravenous glucose infusion (9).

The circulating amino acid concentration is also a potent regulator of hepatic gluconeogenesis (10–13). The importance of this regulatory mechanism can be estimated from data obtained utilizing liver slices (13). These data predict that a threefold increase in the plasma alanine concentration, which has been shown to follow a 5 g high protein meal in rats (14) could result in a 172% increase in the rate of gluconeogenesis (13). Since insulin affects plasma amino acid levels *in vivo*, it is of interest to determine if the hepatic response to alterations in substrate availability is affected by physiological insulin concentrations.

Methods. Animals. Liver donors were male white rats (Charles River) fasted for 22 ± 2.5 hr before use and weighing 85–190 g at the time of use.

Liver Perfusion. Rats were weighed and anesthetized by intraperitoneal injection of 6 mg sodium pentobarbital per 100 g body weight. The liver was perfused by the isolated, *in situ* technique described by Mortimore (15). In all perfusions a nonrecirculating medium was utilized.

The perfusate consisted of canine erythrocytes (hematocrit 26 ± 2%) suspended in Krebs–Ringer bicarbonate solution containing bovine serum albumin (40 g per liter). The final perfusate also contained glucose (5 m*M*) alanine (varied 0.45–10 m*M*), tracer U1-^{14}C-alanine (20 μci/liter, ICN), and palmitate sufficient to yield a total free fatty acid concentration of 600 μeq/liter. The red cells were obtained by cardiac puncture or femoral vein cannulation and were collected in standard citric acid–sodium citrate–dextrose anticoagulant solution. Before use the red cells were washed 5 times with an equal volume of buffered (0.01 *M* phosphate, pH 7.4) isotonic saline and finally with an equal volume of Krebs–Ringer bicarbonate. The perfusate was equilibrated with humidified 95% oxygen—5% carbon dioxide at 37°C for 30–60 min prior to infusion. Glycolysis was negligible during this period as demonstrated by the constancy of the reservoir glucose concen-

tration. The perfusate was infused via the portal vein at a rate of 7 ml per min.

Insulin[1] was added to the inflow perfusate by constant infusion to give concentrations varying from 10–1,000 μU/ml. The infused insulin was in a plasma solution identical to that of the recipient perfusate, but devoid of erythrocytes. In order to measure gluconeogenesis at several insulin concentrations in each liver, a stopcock arrangement was devised in order to change the concentration of the infused insulin solution without interrupting the perfusion. In approximately half of the perfusions the insulin concentration was increased in stepwise fashion, i.e., 10, 200, 1000 μU/ml. In the other half the order of administration was reversed. The gluconeogenic responses did not differ statistically between the two groups.

Preliminary experiments demonstrated that the liver required a period of 20 min for attainment of a new steady state rate of gluconeogenesis following a change in the insulin concentration.

Protocol of the liver perfusion. The surgical procedures were performed as has been described (15). Following surgery, a 30 min perfusion period was allowed for equilibration of the liver before measurements and experimental manipulations were begun. The perfusate was recirculated during this initial period and did not contain tracer ^{14}C-alanine. At the end of 30 min, the perfusion was switched to a nonrecirculating system, insulin infusion was initiated, and tracer-^{14}C-alanine was added to the perfusate. An additional 20 min of nonrecirculating perfusion was performed to allow stabilization of the insulin concentration and of the alanine specific activity in the perfusate entering the liver. Following this period, samples of venous perfusate were taken directly from the outflow cannula at 5 min intervals for 10–15 min. A change in the insulin concentration was always followed by a 20 min stabilization period before sampling was reinitiated. The samples were held at 4°C until analyzed. The results were averaged to give the rates of gluconeogenesis and glucose transfer at that insulin concentration in that liver.

During the perfusion, liver viability was assessed by monitoring hepatic oxygen consumption, perfusion pressure, and bile production. Perfusions were terminated when decreases in the rate of oxygen consumption or bile production or increases in perfusion pressure were observed.

Analysis of Medium. The perfusate glucose concentration was determined by the ferricyanide technique (16) using the Technicon Autoanalyzer. ^{14}C-glucose determinations were made on perfusate plasma following deproteinization with $Ba(OH)_2$-$ZnSO_4$. ^{14}C-glucose was isolated from ^{14}C-alanine either by thin-layer chromatography (13) or by a modification of the resin treatment described by Exton and Park (10). This resin treatment removed more than 98% of the labeled alanine from the sample and provided comparable results to those obtained with the thin-layer chromatography technique. Calculation of the rates of glucose and ^{14}C-glucose production were carried out as described by Mallette *et al.* (11). Production rates are expressed as micromoles per hr per g wet wt of liver.

Results. Effect of insulin on gluconeogenesis from alanine and net glucose release. The rate of glucose production from alanine was found to be a decreasing linear function of perfusate insulin concentration in the range of 10–500 μU/ml, Fig. 1. This relationship is described by the linear regression equation:

$$\text{Rate of Gluconeogenesis } (\mu\text{m/hr/g wet wt}) = 15.93 - 0.0084(\text{Insulin, } \mu\text{U/ml}).$$

Both the slope and the intercept are significantly different from zero ($p < .001$). The rate of gluconeogenesis approached maximal inhibition at an insulin concentration of 500 μU/ml. This is demonstrated by the fact that there was no further change in the rate of gluconeogenesis at an insulin concentration of 1,000 μU/ml.

The effect of increasing insulin concentrations on net hepatic glucose release is shown in Fig. 2. Hepatic glucose release was sig-

[1] The insulin used in these experiments was a gift from J. M. McGuire of the Lilly Research Laboratories.

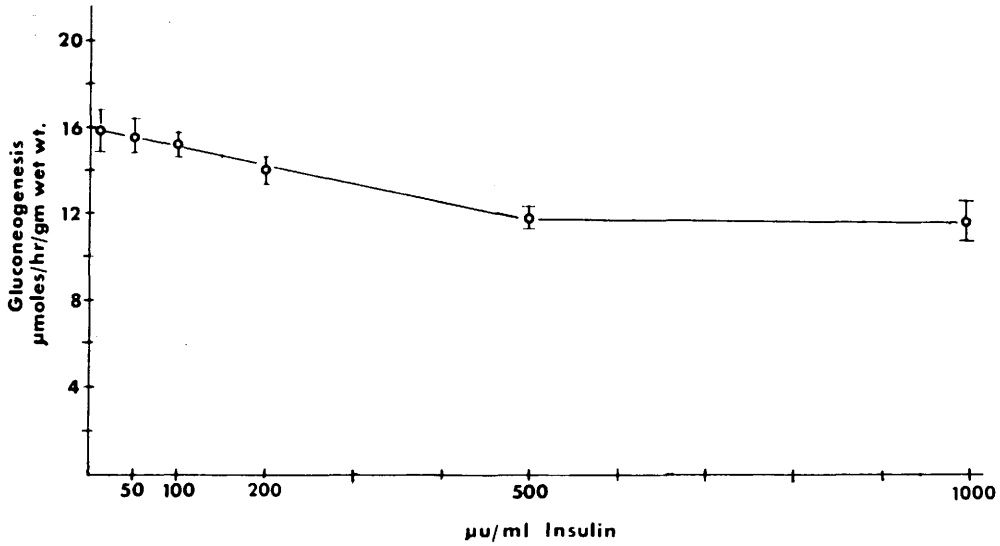

FIG. 1. Rate of gluconeogenesis as a function of the perfusate insulin concentration: Rate of Gluconeogenesis = 15.93 − .0084 (μU/ml insulin); ($t = 4.16$, $p < .001$). Each point represents the mean of four livers. Brackets indicate standard error. The alanine concentration in these perfusions was 2.8 m*M*.

nificantly decreased by physiological increases in the perfusate insulin concentration ($p < .025$).

Relationship between alanine and insulin concentration in determining the rate of gluconeogenesis. Gluconeogenic pathways in perfused rat livers approach substrate saturation only at alanine concentrations

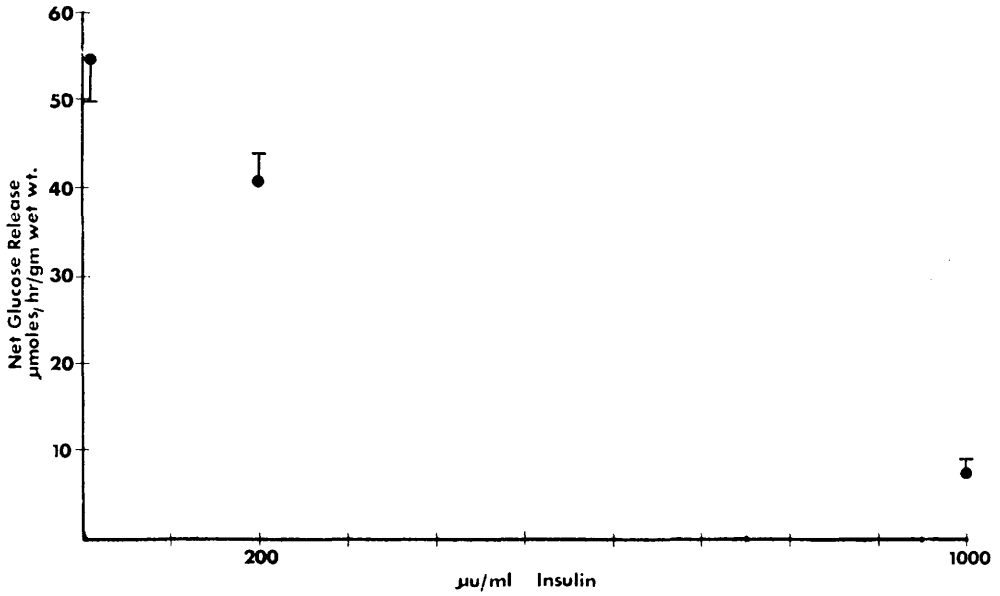

FIG. 2. The rate of net glucose release as a function of the perfusate insulin concentration. The arterial perfusate alanine concentration in these experiments was 2.8 m*M*. Each point represents the mean of 3 livers. Brackets indicate standard error of the mean.

20× the normal plasma concentration (11). In light of the ability of insulin at physiological concentrations to inhibit gluconeogenesis, it was of interest to determine if insulin also altered the capacity of the liver to respond to increased substrate levels. As shown in Fig. 3, the rate of gluconeogenesis is a linear function of the alanine concentration in the range from 0.45–4.0 m*M*. At an insulin concentration of 10 μU/ml this relationship is described by the equation.

Gluconeogenesis (μm/hr/g liver) = 0.61 + 5.53 (Alanine, m*M*) ($t = 6.93$, $p < .001$). Increasing the insulin concentration to 200 or 500 μU/ml significantly decreased the mean rate of gluconeogenesis in this range of alanine concentrations ($p < .01$, by covariance analysis), but did not change the slope significantly. Even at a saturating substrate concentration, 10 m*M* alanine, there was a significant decrease in gluconeogenesis when the insulin concentration was increased from 10–500 μU/ml ($p < .05$).

Discussion. Inhibition of gluconeogenesis by insulin. The present study has demonstrated that the rate of gluconeogenesis from alanine is significantly inhibited by insulin at concentrations which occur *in vivo*. Thus, insulin's inhibitory action on gluconeogenesis accounts for a portion of the insulin effect on hepatic glucose exchange. As shown in Fig. 2, increases in the insulin concentration from 10–1000 μU/ml result in a decrease in net glucose release of 45

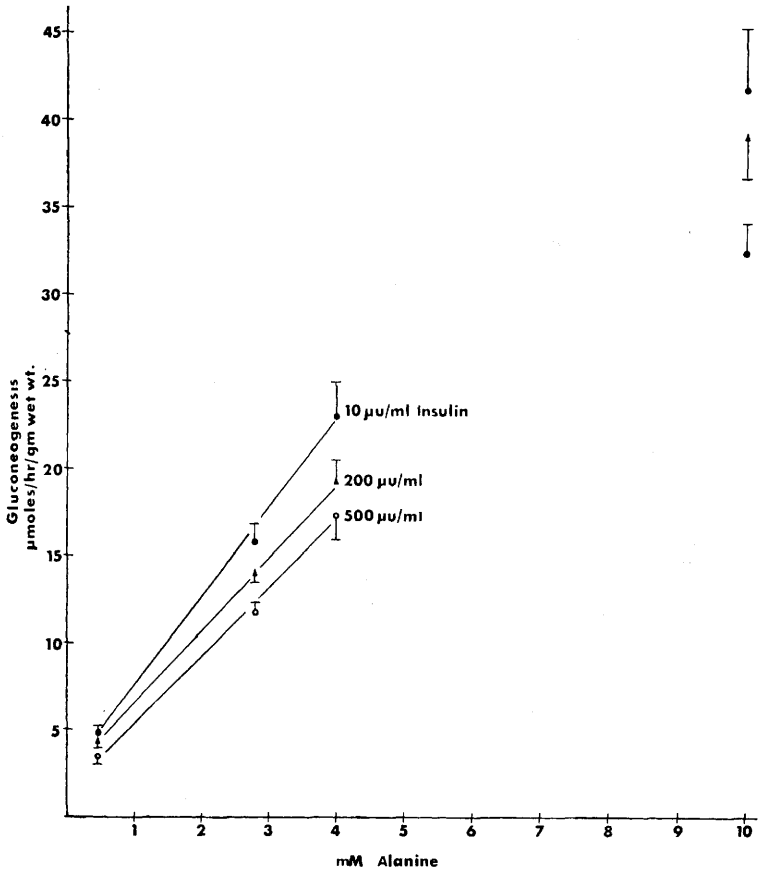

FIG. 3. The effect of physiological concentrations of insulin on the rate of gluconeogenesis as a function of the perfusate alanine concentration. Each point represents the mean of 4 livers. Brackets indicate standard error of the mean.

μmoles/hr/g liver of which the decrease in the rate of gluconeogenesis would account for 4.2 μmoles/hr/g liver, or 9.5% of the total. Thus, the increased rate of gluconeogenesis seen in insulin deficiency may result from the combined effects of uninhibited hepatic gluconeogenesis and increased substrate supply due to a decrease in the peripheral action of insulin (17, 18).

In light of the established ability of insulin to increase net glycogen storage (19), the possibility exists that the observed insulin induced reduction in labeled glucose release represented an increase in storage of the newly formed glucose as glycogen rather than an actual inhibition of new glucose production. This possibility is unlikely in light of the findings of Jefferson *et al.* (7). These investigators observed a significant inhibition of gluconeogenesis by insulin without increased labeling of glycogen. Thus, the insulin inhibition of hepatic release of new glucose is at least in part a result of direct inhibition of gluconeogenesis rather than due exclusively to stimulation of glycogen storage.

Effect of substrate concentration on gluconeogenic response to insulin. In the present study the observed effect of exogenous alanine concentration upon the rate of gluconeogenesis from that substrate closely parallels the effect observed previously in rat liver slices (13). Increasing the insulin concentration lowered the rate of gluconeogenesis at each alanine concentration, but did not significantly alter the ability of the perfused liver to respond to fluctuations in the substrate concentration. In spite of this apparent lack of interaction between alanine and insulin in regulating gluconeogenesis interplay between these two gluconeogenic regulators is important in terms of protein conservation. For example, significant changes in both plasma alanine and insulin concentrations occur subsequent to eating or starving. The threefold increase in portal venous alanine which occurs following ingestion of a 5 g high protein meal in rats (14) would result in a doubling of the rate of gluconeogenesis (Fig. 3). However, the concurrent rise in portal venous insulin which would follow ingestion of the meal reduces gluconeogenesis, thereby conserving protein which would otherwise have been converted to glucose. The conservation of dietary protein by insulin is also enhanced by the stimulation of amino acid uptake and protein synthesis (17, 18, 20).

Summary. The rate of gluconeogenesis from U1-^{14}C-alanine was determined at physiological insulin concentrations in the isolated rat liver perfused with a non-recirculating medium. There was a significant negative relationship between gluconeogenesis and the insulin concentration over the range of 10–500 μU/ml. Increasing the insulin concentration to 1000 μU/ml did not result in any further reduction of gluconeogenesis. Insulin did not alter the gluconeogenic response of the liver to increasing substrate concentrations in these perfusions.

1. Burton, S. D., and Ishida, T., Amer. J. Physiol. **209**, 1145 (1965).

2. Burton, S. D., Mondon, C. E., and Ishida, T., Amer. J. Physiol. **212**, 261 (1967).

3. Glinsmann, W. H., and Mortimore, G. E., Amer. J. Physiol. **215**, 553 (1968).

4. Mackrell, D. J., and Sokal, J. E., Diabetes **18**, 724 (1969).

5. Mortimore, G. E., Amer. J. Physiol. **204**, 699 (1963).

6. Williams, T. F., Exton, J. H., Friedman, N., and Park, C. R., Amer. J. Physiol. **221**, 1645 (1971).

7. Jefferson, L. S., Exton, J. H., Butcher, R. W., Sutherland, E. W., and Park, C. R., J. Biol. Chem. **243**, 1031 (1968).

8. Rudorff, K. H., Windeck, R., and Staib, W., "Regulation of Gluconeogenesis," p. 237. New York, Academic Press, (1971).

9. Blackard, W. G., and Nelson, N. C., Diabetes **19**, 302 (1970).

10. Exton, J. H., and Park, C. R., J. Biol. Chem. **242**, 2622 (1967).

11. Mallette, L. E., Exton, J. H., and Park, C. R., J. Biol. Chem. **244**, 5713 (1969).

12. Ruderman, N. B., and Herrera, M. G., Amer. J. Physiol. **214**, 1346 (1968).

13. Sladek, C. D., and Snarr, J. F., Proc. Soc. Exp. Biol. Med. **138**, 181 (1971).

14. Wheeler, P., and Morgan, A. F., J. Nutr. **64**, 137 (1958).

15. Mortimore, G. E., Amer. J. Physiol. **200**, 1315 (1961).

16. Hoffman, W. S., J. Biol. Chem. **120**, 51 (1937).

17. Carlsten, A., Hallgren, B., Jagenburg, E., Svanborg, A., and Werko, L., Acta Med. Scand. **179**, 361 (1966).

18. Luck, J. M., Morrison, G., and Wilbur, L. F., J. Biol. Chem. **77**, 151 (1928).

19. Mortimore, G. E., King, E., Jr., Mondon, C. E., and Glinsmann, W. H., Amer. J. Physiol. **212**, 179 (1967).

20. Wool, I. G., and Krahl, M. E., Amer. J. Physiol. **196**, 961 (1959).

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