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Glucagon Stimulation of Hepatic Gluconeogenesis in Neonatal Pigs (39078)

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We have previously reported (1) markedly reduced hepatic gluconeogenic capacities from lactate and alanine in piglets during the first 5 days of life. The reason for the low capacities at 5 days of age is not clear, since adequate enzymatic activity in the gluconeogenic pathway appears to be present (1, 2). Increased hepatic fatty acid oxidation had no effect on gluconeogenesis from lactate or alanine in the 5-day-old animals (1).

The present study was performed to determine if an *in vivo* infusion of glucagon would stimulate gluconeogenesis from lactate in livers from 5-day-old piglets. Glucagon was selected because it is known to stimulate gluconeogenesis in rat liver (3), most likely by increasing the conversion of oxaloacetate to phosphoenolpyruvate (PEP) (4). Although glucagon is known to stimulate cAMP accumulation (5), the mechanism by which glucagon or cAMP act on the conversion of oxaloacetate to PEP is currently not known.

Materials and methods. Five-day-old piglets (Yorkshire–Hampshire crosses) of both sexes were obtained from the Swine Research Farm at Michigan State University. The animals were removed from the sows at 4 days of age and placed for 24 hr in a stainless steel cage with overhead heaters and a water source. Control and study animals were randomly selected. Polyethylene catheters (PE 50) were placed in a femoral artery and vein under light nitrous oxide and oxygen anesthesia, and the animals were then placed in comfort slings under radiant heaters and allowed to stabilize. No form of anesthesia was used after the insertion of the catheters. Glucagon (Eli Lilly—Lot no. 69D-87-C) was diluted in 0.2 *M* glycine buffer and frozen in plastic tubes until used. The study animal was infused with glucagon in 0.45% saline at the rate of 100 ng/kg/min for 3 hours, while the control animals received an equal volume, approximately 10 cc, of 0.45% saline. Blood samples for glucose determination were taken at 0, 15, 30, 60, 90, 120, 150, and 180 minutes from start of the infusion, and plasma samples for free fatty acid determination were obtained at 0, 60, 120, and 180 min. The total volume of blood removed never exceeded 3 ml. At the termination of the infusion, the animals were killed by cervical dislocation, the livers were rapidly removed, and thin hand-cut slices, weighing approximately 100 mg, were made using a Stadie–Riggs microtome. Slices were placed in 15 ml of Krebs–Ringer buffer and 10 m*M* L-lactate-3-^{14}C. The incubation conditions and method for glucose determination have been described previously (1). Blood glucose was determined with glucose oxidase (Worthington Biochemical), and plasma free fatty acid was determined by a modified Novak method (6).

Blood glucose and liver slice data were analyzed by analysis of variance. Regression equations were generated for the liver slice data and compared by an *F* test between two regression coefficients (7). All data are expressed as mean ± SEM.

Results. The infusion of glucagon did not significantly change the blood glucose (Fig. 1) or the plasma free fatty acid levels (Table I) in the study animals as compared to control animals.

The results of the *in vivo* infusion of glucagon on the capacity of liver slices to generate glucose from lactate are shown in Table II. For each liver, duplicate flasks were incubated for 0.5, 1, and 1.5 hours, and the gluconeogenic rate was calculated from the new glucose data. The equation derived for the control group is $y = 9.60x - 0.166$, where y = glucose and x = time, and that for the glucagon treated group is y =

$13.04x - 0.538$. The slope of these lines is significantly ($P < 0.001$) different from zero. The slope of the equation describing gluconeogenic activities for the three time periods was significantly greater ($P < 0.05$) in glucagon infused animals than in control animals.

Discussion. The establishment of hepatic gluconeogenesis in nonruminant mammalian newborns appears to be dependent on induction of phosphoenol pyruvate carboxykinase (PEPCK) activity following birth. *In utero* injections of glucagon, adrenalin, and noradrenalin induced PEPCK activity in rat fetuses as does premature delivery (8). During the first day of life, human serum glucagon levels have been reported both to remain unchanged (9) and to increase (10), with no correlation to blood glucose values. In newborn rats, serum glucagon levels increase (10, 11), though *in vitro* pancreatic secretion of glucagon has no correlation to glucose concentration (10). In human infants, intravenous infusions of glucagon have little effect on plasma amino acid levels until 3 days of age (12). At 3 days of age, infusions of glucagon produced a fall in plasma amino acid levels similar to that found in adults (13). Therefore, there is indirect evidence for glucagon stimulation of gluconeogenesis in the infant, but there is no evidence that glucagon stimulates flow of substrate in the gluconeogenic pathway.

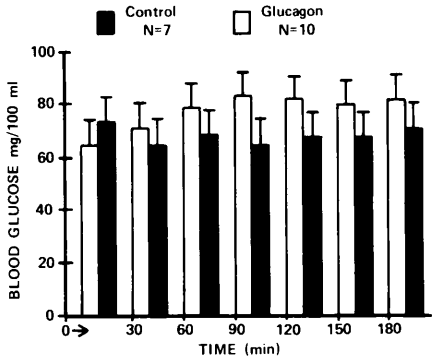

FIG. 1. Blood glucose concentration during infusions of saline (control) and glucagon in 5-day-old piglets. The bars represent mean ± SEM.

In our study, a 3-hr infusion of glucagon at 100 ng/kg/min produced a significant increase in the gluconeogenic capacity from lactate by piglet liver slices. However, the glucagon infusion did not produce a significant difference in blood glucose or plasma free fatty acid values between the study and

TABLE I. EFFECT OF GLUCAGON ON PLASMA FREE FATTY ACID.

Treatment	Time (min) 0	60	120	180
Control ($n = 3$)	0.29 ± 0.07[a, b]	0.36 ± 0.03	0.24 ± 0.08	0.37 ± 0.02
Glucagon ($n = 3$)	0.37 ± 0.11	0.34 ± 0.12	0.25 ± 0.06	0.29 ± 0.06

[a] Plasma FFA is expressed as μeq/ml.
[b] Values presented are means ± SEM.

TABLE II. EFFECT OF GLUCAGON ON CONVERSION OF LACTATE TO GLUCOSE BY LIVER SLICES FROM 5-DAY-OLD PIGS.

Treatment	Incubation time (hours) 0.5	1	1.5
Control ($n = 7$)	3.7 ± 0.66[a] (moles glucose $g^{-1}hr^{-1}$)	11.24 ± 2.1	13.34 ± 2.3
Glucagon ($n = 10$)	5.80 ± 1.1	15.11 ± 3.2	18.57 ± 5.8

[a] Value pressented are means ± SEM.

control animals. The failure to demonstrate the expected rise in plasma free fatty acid values following the glucagon infusion may be due to deficient quantities of white adipose tissue (14), rather than a failure of glucagon to stimulate lipolysis and release of free fatty acids. It is also possible that the infused glucagon stimulated the release of insulin which in turn suppressed peripheral lipolysis and release of free fatty acids, without effecting hepatic gluconeogenesis.

Our results suggest that the naturally occurring glucose homeostatic responses to fasting in the piglet can be further amplified by exogenous glucagon. We have not studied, nor are we aware of studies on the plasma glucagon response to fasting in the piglet, but it is clear in adult man, that plasma glucagon levels rise in response to fasting (13, 15).

In rat liver cells, glucagon stimulates gluconeogenesis after PEP formation (16), as well as at the step involving the conversion of oxaloacetic acid to PEP (4). The stimulation after PEP appears to be due to the enhancement by glucagon of substrate flow through fructose-1, 6-diphosphatase and the simultaneous inhibition of substrate flow through phosphofructokinase (17, 18). The effect on conversion of oxaloacetate to PEP is most likely due to stimulation of PEPCK activity (4). Such effects would promote glucose synthesis from lactate and could be the basis for the enhancement of glucose production from lactate by glucagon in piglet liver slices.

It has been shown that glucagon can stimulate PEPCK synthesis in livers of newborn rats (19). However, it is unlikely in this study that enhancement of glucose production was due to synthesis of new enzyme since the effect was apparent after a brief infusion. In rat livers glucagon has been shown to stimulate the conversion of oxaloacetate to PEP after $2\frac{1}{2}$ min of perfusion (4). Our data do not permit us to conclude which enzyme or enzymes of gluconeogenesis were affected by the glucagon infusion.

Summary. The effect of an infusion of glucagon on gluconeogenesis was studied in 5-day-old piglets. Glucagon stimulated hepatic new glucose formation from lactate, but did not significantly change blood glucose or plasma free fatty acid levels. The data suggest that glucagon enhances substrate flow in the gluconeogenic pathway in neonatal animals.

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