A STIMULATED RATE OF GLUCONEOGENESIS IN PERFUSED LIVERS OF HYPOPHYSECTOMIZED RATS*

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Summary

Production of glucose and urea by livers of hypophysectomized rats was twice that by livers of normal rats when both were perfused with medium containing amino acids at either one or ten times their normal plasma concentrations. This suggested a stimulated rate of gluconeogenesis in the livers of hypophysectomized rats. Increased rates of conversion of ^{14}C-alanine and ^{14}C-lactate to glucose were observed in livers of these rats.

Studies with the perfused rat liver demonstrated that amino acid catabolism was increased following hypophysectomy (1). Since gluconeogenesis is an important catabolic pathway for many amino acids, it is possible that hypophysectomy caused an increased conversion of amino acids to glucose. While there is a substantial amount of evidence indicating that hypophysectomy would diminish hepatic gluconeogenesis (2-6), hypophysectomized (hypox) animals do maintain normal levels of blood glucose in the face of above normal rates of carbohydrate utilization (7-9).

In the present study the gluconeogenic capacity of the liver was found to be increased following hypophysectomy. Hypophysectomy resulted in an increased synthesis of glucose from both alanine and lactate in the perfused rat liver.

Materials and Methods

Normal and hypox male rats were fed regular laboratory chow and water *ad libitum* up to time of sacrifice. Hypox rats were 14-16 days postoperative and all rats weighed 95-100 g at time of use.

Livers were perfused *in situ* for 60 min. at 37° by the technique described by Mortimore (10). The basic perfusion medium consisted of Krebs-Henseleit bicarbonate buffer (11) containing 3% serum albumin, 10 mM glucose and washed sheep erythrocytes to give a hematocrit of 20%.

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The initial recirculating volume of perfusate was 50 ml from which 3 ml samples were taken at 15, 30 and 60 min. after the start of perfusion. At the end of perfusion, the livers were quickly removed and frozen in a Wollenberger clamp at the temperature of liquid nitrogen (12).

Perfusate and blood plasma samples were analyzed for glucose (13), urea (14) and ^{14}C-glucose (15). Glycogen was extracted from frozen liver samples (16) and the glucose was estimated following acid hydrolysis (13). The ^{14}C-glucose present in glycogen was determined (15). Glycogen levels are reported as glucose equivalents per g wet liver weight.

Results

Hypophysectomy reduced the liver to body weight ratio by 16%, reduced hepatic glycogen content by one-half and increased plasma urea levels by 100%, but had no effect on the level of blood glucose (Table I).

Table I

EFFECTS OF HYPOPHYSECTOMY OBSERVED IN VIVO

	Condition of Rats	
	Normal	Hypox
Liver Weight (g/100 g body wt.)	5.17 ± .12 (9)	4.34[b] ± .18 (9)
Liver Glycogen Content (μmoles glucose/g)	312 ± 13 (14)	158[a] ± 30 (13)
Plasma Glucose Concentration (mM)	8.56 ± .28 (10)	8.48 ± .42 (8)
Plasma Urea Concentration (mM)	5.42 ± .33 (10)	11.02[a] ± .65 (15)

Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital, their abdominal cavities exposed and aortic blood drawn into chilled heparinized tubes. Immediately following collection of the blood sample, the livers were quickly excised and frozen in Wollenberger clamps at the temperature of liquid nitrogen (12). Liver and blood plasma samples were stored at −70°C for subsequent analysis as described in the text. Mean values are presented followed by the standard error of the mean. The number of observations is indicated in parenthesis.

[a]differs from Normal by $P < 0.001$

[b]differs from Normal by $P < 0.005$

When livers were perfused with medium containing amino acids at normal plasma concentrations (Table II), production of both glucose and urea by livers of hypox rats was increased 2 to 4-fold. When medium amino acid levels were raised to ten times their normal plasma concentrations, both urea and glucose production increased 2 to 3-fold, but these rates still were

Table II

EFFECTS OF HYPOPHYSECTOMY OBSERVED IN THE PERFUSED RAT LIVER

Condition of rats	Substrate	Net Glucose Production (μmoles/g liver/h)	Urea Production (μmoles/g liver/h)	Final Glycogen Content (μmoles glucose/g liver)
Normal (6)	1 X AA	11.61 ± 2.22	9.75 ± 1.68	–
Hypox (6)		33.11[c] ± 5.50	21.86[a] ± 1.93	–
Normal (10)	10 X AA	27.94 ± 3.83	32.00 ± 3.36	208 ± 18
Hypox (6)		67.11[c] ± 12.06	67.54[a] ± 7.75	108[b] ± 11
Normal (12)	Alanine (10 mM)	21.28 ± 2.98	16.68 ± .79	267 ± 15
Hypox (18)		41.44[a] ± 2.72	36.46[a] ± 1.36	88[a] ± 11
Normal (17)	Lactate (10 mM)	30.89 ± 2.22	8.14 ± .39	253 ± 15
Hypox (6)		71.17[a] ± 6.72	12.29[a] ± .79	142[d] ± 37

Amino acids were added to the basic perfusion medium to give initial perfusate concentrations approximating the amino acid composition of serum from normal fed rats (1 X AA) or ten times that amount for each amino acid (10 X AA) (17). With alanine as the primary gluconeogenic substrate, the basic perfusing medium contained unlabeled L-alanine at an initial concentration of 10 mM, 0.025 μCi/ml of L-alanine-U-^{14}C and the remaining 19 L-amino acids at their normal plasma concentrations. For the lactate gluconeogenesis experiments the basic perfusion medium contained 10 mM L-lactic acid (sodium salt) and 0.02μCi/ml DL-lactic acid-2-^{14}C.

Mean values are presented followed by the standard error of the mean. The number of observations is indicated in parenthesis. All values are expressed per g wet liver weight.

[a]differs from normal by $P < 0.001$ [c]differs from normal by $P < 0.01$
[b]differs from normal by $P < 0.005$ [d]differs from normal by $P < 0.02$

greater in livers of hypox rats. These data suggested a stimulation of amino acid utilization and gluconeogenesis in the livers of these animals.

Conversion of alanine and lactate to glucose was investigated using saturating levels of labeled substrates (Table II, Figure 1). With alanine as substrate net glucose production was increased 2-fold in livers of hypox rats. With lactate as substrate net glucose production was increased in both groups, but production by livers of hypox rats still was significantly greater. As expected urea production was increased with alanine as substrate, but with either substrate production was greater in livers of hypox rats. In all groups liver glycogen content fell from pre-perfusion levels and contributed to glucose production. On the average, however, net glycogen breakdown appeared to be greater in normal livers, making it unlikely that the increased net

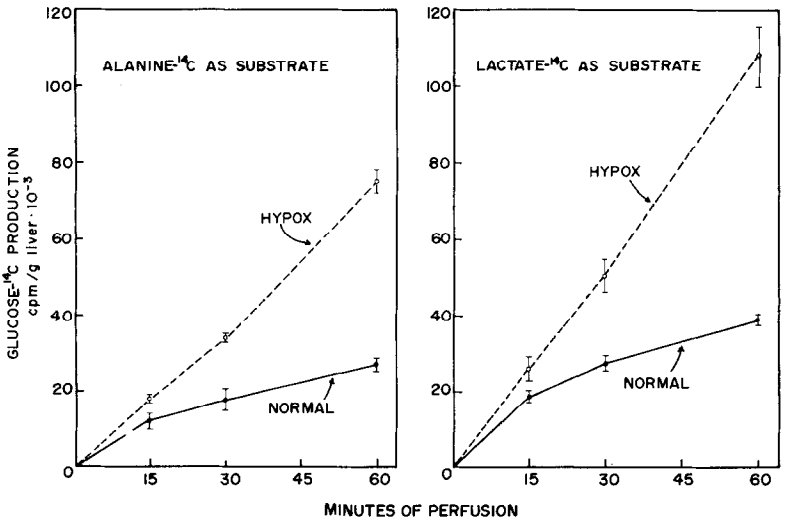

Figure 1. Conversion of ^{14}C-alanine and ^{14}C-lactate to ^{14}C-glucose by perfused livers of normal and hypophysectomized rats. Each point represents the mean of 6 to 18 observations. The vertical bars represent the standard error of the mean. Details of the perfusion system and medium are given in the Methods and Materials section and in the legend to Table II.

glucose production by the livers of hypox rats resulted from increased glycogenolysis.

Glucose arising from conversion of substrates by the gluconeogenic pathway was distinguished from glucose derived from glycogenolysis by estimating conversion of labeled substrate to labeled glucose. As seen in Figure 1, synthesis of ^{14}C-glucose from either ^{14}C-alanine or ^{14}C-lactate was greatly stimulated in the livers of hypox rats. Production of ^{14}C-glucose by control livers leveled off whereas production by livers of hypox rats remained linear during the entire perfusion period.

Discussion

The present results demonstrate that the gluconeogenic capacity of the liver was increased 3 to 4-fold following hypophysectomy. The reactions involved in this effect are unknown at present. Increased ability of the hypox animal to convert amino acids and other precursors to glucose has physiological importance in that it enables the animal to maintain a normal level of blood glucose in the face of above normal rates of glucose utilization (7-9). In addition, the impaired ability of the hypox animal to mobilize free fatty acids (18) emphasizes the necessity of additional glucose to maintain energy metabolism. In other experiments, plasma levels of several gluconeogenic amino acids, particularly alanine, were found to be reduced following hypophysectomy suggesting that amino acids provide an important source for the increased glucose synthesis.

Hepatic gluconeogenesis is known to be regulated by several hormones, fatty acids and by the supply of glucose precursors to the liver (19). A number of changes in hormone and substrate levels in hypox animals would tend to reduce the rate of gluconeogenesis. These include low levels of thyroid and adrenocortical hormones and low concentrations of amino acids, fatty acids and glycerol. The possibility that gluconeogenesis was reduced was suggested by some preliminary findings (19) and by results of earlier studies (2-6). One might argue that the hypox animal is partially fasted and that this is responsible for the increased gluconeogenesis. However, the hypox animal has impaired ability to provide the usual stimuli to gluconeogenesis that are associated with fasting. These include elevated levels of fatty acids (20), amino acids and glucocorticoids (21). Insulin is known to inhibit gluconeogenesis (22) and it would seem possible that the decreased plasma level of this hormone following hypophysectomy (23) could contribute to the stimulation of the gluconeogenic pathway. Insulin is thought to affect gluconeogenesis through changes in levels of cyclic AMP. Since glucocorticoids are required for this effect, the role of cyclic AMP in the livers of hypox rats is uncertain. Effects of insulin and cyclic AMP on gluconeogenesis in the hypox rat and reactions involved in the stimulation are currently under investigation.

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