CHANGES IN HEPATIC FATTY ACID SYNTHESIS FOLLOWING GLUCAGON INJECTIONS IN VIVO[1,2]

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SUMMARY A marked decrease in hepatic fatty acid synthesis from glucose was observed fifteen and thirty minutes after intravenous injection of glucagon (1mg/kg) in anesthetized rats. This effect was apparently brought about by an inhibition of acetyl CoA carboxylase activity. Acitivities of fatty acid synthetase, citrate cleavage enzyme, NADP-malic-, glucose-6-phosphate-, and isocitric dehydrogenase were not affected by glucagon.

Introduction

Available evidence indicates that glucagon markedly depresses fatty acid synthesis. In this respect, Haugaard and Stadie (1) observed that glucagon decreased the incorporation of acetate-1-^{14}C into fatty acids in rat liver slices, and Berthet (2) reported in similar effect of glucagon on fatty acid synthesis in rabbit liver preparations. A decrease in fatty acid synthesis from acetate-2-^{14}C in response to glucagon has also been observed in the isolated perfused rat liver (3,4). A recent report from this laboratory demonstrated a gradual inhibition of both fatty acid synthesis from glucose-U-^{14}C and glucose oxidation by rat liver slices as the concentration of glucagon was increased in the incubation media (5).

1) The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

2) In conducting research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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The mechanisms by which glucagon inhibits fatty acid synthesis remains obscure. Since, glucagon decreases activities of the key hepatic glycolytic, and stimulates the key gluconeogenic enzymes (6), it would appear that the hormone could exert the control over the fatty acid synthetic pathway by affecting activities of specific enzymes. However, this possibility remains to be verified.

Consequently, in the present study we examined a vivo glucagon effects upon the activities of several enzymes associated with hepatic fatty acid synthesis. In addition, oxidation of glucose-U-^{14}C and its incorporation into fatty acids and glycerol by hepatic tissue slices were determined.

Experimental

Male Holtzman rats ranging in weight from 250-300 gm were individually housed at 25^{o}C and fed a purified diet for seven days before experimentation. The diet contained by weight the following components: 18% casein, 75% sucrose, 3% corn oil, 4% USP mixture XIV, and a complete vitamin supplement. Food as well as water were available ad libitum.

The animals were anesthetized with 50 mg/kg pentobarbital i.p., and ten minutes later the abdomen was opened. Approximately 500 mg of hepatic tissue was removed (zero time) and cooled in ice-cold saline. A small piece of gauze was placed over the liver incision site for hemostasis.

Glucagon was injected into the inferior vena cava below the renal veins in a dosage 1 mg/kg BW, after collecting a small volume of venous blood in a heparinized syringe for plasma glucose determination. Crystalline glucagon, obtained from Eli Lilly & Co., was dissolved in the manufacturer's diluent, which contained 1.6% glycerol and 0.2% phenol (1 mg glucagon/ml diluent). A gauze was placed over the exposed abdomen and kept moist with saline.

Three experiments were conducted as follows:

Experiment 1. Five and fifteen minutes after glucagon injection,

TABLE 1

EFFECT OF GLUCAGON ON THE ACTIVITIES OF RAT HEPATIC ENZYMES AND ON GLUCOSE UTILIZATION BY HEPATIC TISSUE (EXP. 1).

	TIME AFTER INJECTION (MIN)		
ENZYME[1]	0	5	15
Glucose-6-phosphate dehydrogenase	171.2± 20.3	150.4± 9.3	150.3 ± 16.4
NADP-malic dehydrogenase	109.7 ± 13.6	102.4 ± 8.2	105.4 ± 16.7
Isocitric dehydrogenase	1,228.6 ± 69.8	1,463.7 ± 183.0	1,209.5 ± 70.4
Citrate cleavage	64.1 ± 8.3	52.5 ± 3.7	67.8 ± 11.8
METABOLITE[2]			
CO_2	2,171.3 ± 199.6		1,554.3 ± 165.6*
Fatty acids	609.8 ± 64.2		218.1 ± 15.3*

1) Values are mean ± SE from 5-7 rats nanomoles product/min/mg protein

2) Values are mean ± SE μmoles glucose incorporated/2 hr/gm wet weight

* Indicates significant difference from zero time, $P<0.05$.

additional liver samples were collected. A blood sample was also taken at 30 min from the inferior vena cava for plasma glucose determination. One portion of the liver sample was homogenized according to Muto and Gibson (7) and the soluble liver supernatant obtained following centrifugation at 105,000 x g for 30 minutes and used for the assay of the following enzymes: glucose-6-phosphate dehydrogenase (8) NADP-malic-, and isocitric dehydrogenase (9), and citrate cleavage enzyme (10).

Tissue slices prepared from the second portion of the liver were

incubated for 2 hours at 37°C, under 95% O_2-5% CO_2, in 25 ml Erlenmeyer flasks. The flasks contained 3.0 ml of Kreb-Ringer bicarbonate buffer, 30 μmoles glucose and 0.25 μCi glucose -U-^{14}C. At the end of the incubation period, the substrate incorporation into fatty acids and $^{14}CO_2$ was measured by the previously described methods (11,12). Protein was determined by the method of Lowry et al. (13), and plasma glucose was measured by the glucose oxidase procedure (14).

Experiment 2. Liver samples were collected fifteen and thirty minues after glucagon injection and were again divided into two pieces. One was used for the assays of fatty acid synthetase and acetyl CoA carboxylase accoring to the methods of Hsu et al. (15) and Majerus et al. (16), respectively. The second piece was used to determine glucose-U-^{14}C incorporation into the hepatic tissue metabolites as described under Experiment 1.

Experiment 3. As a control for the foregoing experiments, animals were injected with the glucagon diluent (1 ml/kg) which contained 14 mg/ml of lactose, 1.6% glycerol and 0.2% phenol. The activity of acetyl CoA carboxylase and the incorporation of glucose-U-^{14}C into hepatic fatty acids and $^{14}CO_2$ were determined at time 0, 15 and 30 minutes after injection.

Enzyme activities are expressed as nanomoles of substrate metabolized per minute per milligram of protein, and glucose incorporation into tissue metabolites as micromoles per gram of tissue per 2 hours. The significance of difference between means was calculated by Student's t-test.

Results

The results of Experiment 1, summarized in Table 1, indicate glucagon had no effect on the activities of glucose-6-phosphate-, NADP-malic-, isocitric dehydrogenase and citrate cleavage enzyme. Compared to the preinjection liver sample, glucose oxidation and incorporation into fatty acids fifteen minutes after injection, however, was reduced by 29 and 64%, respectively.

The data obtained in Experiment 2 (Table 2) show that glucagon did not alter the activity of fatty acid synthetase. However, the hormone did reduce the activity of acetyl CoA carboxylase fifteen minutes after injection by about 55%, and at the end of the experimental period by 70%. Thirty minutes after injection, fatty acid synthesis in liver slices was decreased by 74% but glucose oxidation or incorporation into glycerol was not affected by glucagon.

In the control experiment, the glucagon diluent had no significant effect on plasma glucose, acetyl CoA carboxylase activity or on glucose oxidation and incorporation into fatty acids. Plasma glucose levels were 142 ± 6 and 312 ± 7 mg/100 ml at zero time and fifteen minutes, respectively, in Experiment 1; they were 151± 5 and 349 ± 8 mg/100 ml in Experiment 2.

Discussion

The findings in this study are consistent with our previous report concerning *in vitro* inhibitory effect of glucagon on hepatic oxidation of glucose and glucose conversion into fatty acids (5). *In vivo* reduction of fatty acid synthesis was apparent fifteen minutes after injection of glucagon, and the effect persisted at least thirty minutes. In contrast, the glucagon effect on glucose oxidation was less pronounced, persisting for only fifteen minutes.

The inhibition of fatty acid synthesis was accompanied by a parallel reduction in acetyl CoA carboxylase activity. This enzyme appears to be rate-limiting in the regulation of fatty acid synthesis in animal tissues (17,18); its activity is controlled by the tissue enzyme levels (19) and by metabolic modulators (19,20). One of these, long-chain fatty acyl CoA compounds in physiological concentrations has been suggested to be an inhibitor of carboxylase activity (21). Free fatty acids also inhibit the carboxylase in vitro (22). The hepatic concentration of long-chain acyl CoA derivatives is increased by fasting and by high intake of dietary fat (23,24), conditions which decrease fatty acid synthesis (25-27). Thus,

TABLE 2

EFFECT OF GLUCAGON ON FATTY ACID SYNTHETASE, ACETYL CoA CARBOXYLASE AND GLUCOSE UTILIZATION BY HEPATIC TISSUE (EXP. 2).

	TIME AFTER INJECTION (MIN)		
ENZYME[1]	0	15	30
Fatty acid synthetase	26.4 ± 9.7	28.8 ± 8.1	22.1 ± 2.7
Acetyl CoA carboxylase	6.3 ± 1.2	2.8 ± 1.0*	1.9 ± 0.7*
METABOLITE[2]			
CO_2	2,435.6 ± 269.3		2,274.0 ± 382.6
Fatty acids	766.5 ± 80.2		197.3 ± 20.1*
Glycerol	992.3 ± 106.8		697.2 ± 88.7

1) Values are mean ± SE from 5-7 rats nanomoles product/min/mg protein

2) Values mean ± SE μmoles glucose incorporated/2hr/gm wet weight

* Indicates significant difference from zero time, $P<0,05$.

enhanced mobilization of fatty acids from triglyceride store in fasting, fat feeding or diabetes would tend to increase the tissue concentration of long-chain acyl CoA compounds. This would account for the decreased fatty acid synthesis under these conditions of sustained metabolic stress.

Administration of glucagon elicits similar metabolic effects; that is, the hormone stimulates free fatty acid release from adipose tissue in rats (28), and increases hepatic tissue levels of long-chain fatty acyl CoA (29), thus inhibiting the activity of hepatic carboxylase. Alternatively, it has been demonstrated that the carboxylase activity is inhibited by phosphory-

lation of the enzyme protein, which is mediated by ATP-dependent kinase (30). It has been shown that hepatic levels of cyclic AMP are markedly elevated by glucagon (31). Since glucagon action is thought to be mediated by cyclic AMP, it is possible that this nucleotide also mediates the glucagon effect on hepatic acetyl CoA carboxylase. It has been suggested that the metabolic effects of cyclic AMP are mediated through its interaction with regulatory proteins (32), since cyclic AMP exerts its control in bacterial systems by binding with a cyclic AMP - receptor protein (33,34). Hence, it is conceivable that cyclic AMP may regulate the activity of hepatic acetyl CoA carboxylase by interacting with a regulatory protein.

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