KETOGENIC AND LIPOLYTIC EFFECTS OF GLUCAGON ON LIVER*

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Received July 5, 1966

The glycogenolytic effect of glucagon on liver has been extensively investigated. Increased glycogen breakdown and glucose production are apparent after both in vivo and in vitro administration, depending on the activation of phosphorylase, mediated by the formation of adenosine-3'5'-phosphate (cyclic AMP) by the adenyl cyclase system. In addition, glucagon has been shown to stimulate gluconeogenesis in livers of fasted rats (Struck et al., 1965; Garcia et al., 1966). Other actions of glucagon on the liver include inhibition of incorporation of acetate into fatty acids and cholesterol (Berthet, 1958), and of leucine into liver proteins (Pryor and Berthet, 1960) and increased ketogenesis. The ketogenic effect of glucagon is apparent in vivo (Foa and Weinstein, 1951) and in vitro (Haugaard and Haugaard, 1954; Berthet 1958). The present communication reports further experiments on increased ketogenesis due to glucagon, and suggests that the likely mechanism of this effect is activation of hepatic lipase, thus increasing the supply of fatty acids for β-oxidation within the liver.

Materials and Methods

Male Holtzman rats (200-250 g) and male albino mice (20-25 g)

* Supported by grant PHS AM-07211

were used. Glucagon (Lot No. 258-234B-167-1) was kindly supplied by Dr. W. N. Shaw of the Lilly Research Laboratories, Indianapolis. To investigate the in vivo effect, glucagon was injected I.P. (1 mg/kg) 30 minutes before sacrifice. Liver slices and homogenates were incubated for 90 minutes at 37° in Ringer-bicarbonate buffer (pH 7.4) with 95% O_2 and 5% CO_2 as the gas phase. The concentration of glucagon for in vitro studies was 100 µG/ml of incubation medium and cyclic AMP (Sigma) was used at a concentration of 10^{-3} molar. Guinea pig serum containing antibodies to insulin (Robinson & Wright, 1961) was injected I.P.

Various sub-cellular fraction of liver, homogenized in 0.154 M KCl, were prepared (de Duve et al., 1955) and the lipase activity of these fractions was estimated by hydrolsis of endogenous triglycerides or an emulsion of cottonseed oil (trilinolein) in Ringer-bicarbonate buffer, under anaerobic conditions. Ketone bodies were estimated separately by an enzymatic procedure (Williamson et al., 1962) and free fatty acids (FFA) were titrated with alkali (Dole, 1956). Triglycerides were separated by column chromatography from other liver lipids contained in a chloroform: methanol extract, saponified and estimated as fatty acids or glycerol.

Results and Discussion

Ketone production by liver slices from starved rats given glucagon in vivo 30 minutes before sacrifice is shown in Table 1. There was a significant increase in the formation of both acetoacetate and β-hydroxybutyrate by the slices from those rats which had been given glucagon. Similar increases were obtained when chow-fed and fat-fed rats were used.

When glucagon was added in vitro to incubating rat liver slices a small but significant increase in acetoacetate and β-

hydroxybutyrate formation occurred. Ketogenesis by mouse liver homogenates was also stimulated by glucagon *in vitro*, and cyclic AMP *in vitro* led to a more than twofold increase in the production of acetoacetate (Table 1).

Table 1. Ketone Body Production By Liver

		Acetoacetate (μM/gm/90min)	β-Hydroxybutyrate (μM/gm/90 min)
Starved Rat Liver slices	Control	4.00 ± 0.22	2.31 ± 0.17
	Glucagon *in vivo*	4.90 ± 0.36*	3.55 ± 0.20*
Fed Rat Liver Slices	Control	2.28 ± 0.11	1.34 ± 0.09
	Glucagon *in vitro*	2.56 ± 0.10*	1.74 ± 0.15*
Mouse Liver Homogenates	Control	0.35 ± 0.02	N.D.
	Glucagon *in vitro*	0.55 ± 0.04*	N.D.
	Cyclic AMP *in vitro*	0.82 ± 0.14*	N.D.

Values are means + S.E.M. of 6 observations. N.D. = No. determination. *Statistically significant difference from corresponding control ($P < 0.05$).

Since the ketogenic effect of glucagon was apparent *in vitro*, as well as *in vivo*, it seemed possible the fatty acids within the liver were made available for β-oxidation to ketones. This was tested by measuring the rate of increase in tissue free fatty acids during anaerobic incubation of liver homogenates from rats killed 30 minutes after glucagon or control injections. The results are shown in Table 2. The increase in free fatty acids during incubation was greater than the controls in the livers from rats injected

with glucagon. That the probable source of the free fatty acids was the liver triglyceride fraction has been confirmed by estimating the triglyceride content of the homogenates at the beginning and end of incubation (Table 2).

Table 2. Lipolytic Effects of Glucagon on Rat Liver

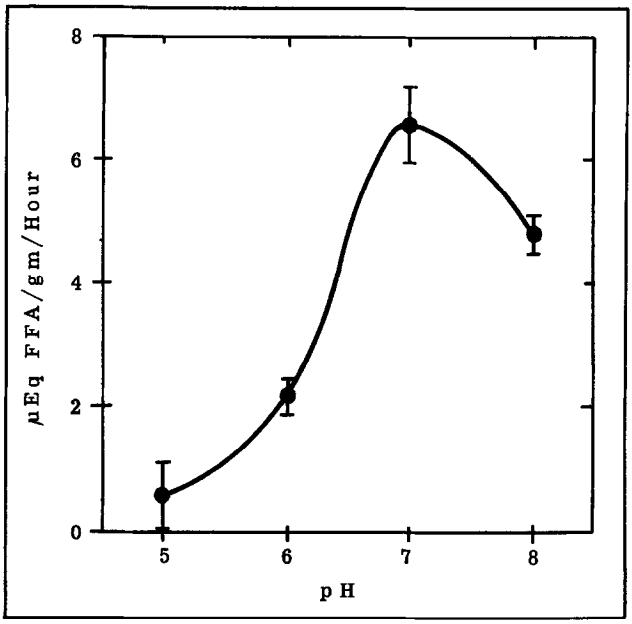
	Tissue Triglycerides		Lipase Activity
	Initial	Net Change	
	μmoles/gm	μmoles/gm/60min.	μEqFFA/gm/60min.
Control	3.97 ± 0.31	-0.56 ± 0.20	1.06 ± 0.20
Glucagon	3.08 ± 0.15	-1.01 ± 0.22	3.80 ± 0.13

Values are means ± S.E.M. of 3 observations for the triglyceride, and mean ± S.E.M. of 5 observations for FFA.

The fall in liver triglyceride concentration was greater in the homogenates from glucagon treated rats. When the livers of rats injected with glucagon or saline were analyzed for triglycerides without incubation, the proportion of liver lipids which was triglyceride in the control animals was 64.1 ± 5.1 μMoles per gram of lipid extracted, whereas the corresponding figure for the glucagon treated rats was 32.3 ± 4.4 (Mean ± S.E.M. of 6 observations).

The liver lipase has been further investigated, and preliminary results are reported below. The activity was similar whether endogenous fat or trilinolein was used as substrate, and was markedly diminished by heating at 80° for 10 minutes. The optimum pH for this enzyme is about 7 (Figure 1).

EFFECT OF pH ON HEPATIC LIPASE ACTIVITY



Values are means ± S.E.M. of 3 observations.

Neither protamine sulfate (150 μG/ml), which inhibits lipoprotein lipase, nor eserine (2.5 μG/ml), which inhibits pancreatic lipase, had any effect on the liver lipase described here. Tolbutamide, which will reduce ketogenesis *in vivo* and *in vitro* (Boshell et al., 1960), depressed hepatic lipase activity to 35% of the control level. The lipase activity in livers removed from rats made insulin deficient by the injection of guinea-pig anti-insulin serum 45 minutes before sacrifice, increased to a rate 52% above that of control livers.

There has been little reference to hepatic lipase in the literature. The experiments described here indicate that such an enzyme exists, and is sensitive to glucagon. It seems likely that the effect of glucagon on ketogenesis depends on activation of the adenyl cyclase system in a manner similar to its glycogen-

olytic effect, except that hepatic lipase, rather than phosphorylase, is activated. The free fatty acids released within the liver, by hydrolysis of triglycerides, form an ester with CoA and are then available for oxidation to ketone bodies. An increase in the liver content of acyl-CoA and acetyl-CoA after glucagon administration has been shown by Williamson (Personal communication). The gluconeogenic action of glucagon may also be a function of the increased content of acyl-CoA and acetyl-CoA esters in these livers, since acyl-CoA esters exert an inhibitory effect on pyruvate decarboxylation, and acetyl-CoA directly stimulates pyruvate carboxylation.

References

Berthet, J., Proc. 4th Int. Cong. Biochem. Vienna. Vol. 15. p. 107, 1958.
Boshell, B. R., Zahnd, G. R., and Renold, A. E., Metabolism 9, 21 (1960)
Dole, V. P., J. Clin. Invest. 35, 150 (1956)
de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. and Appelmans, F., Biochem. J. 60, 604 (1955)
Foa, P. P., and Weinstein, H. R., Fed. Proc. 10, 43 (1951)
Garcia, A., Williamson, J. R., and Cahill, G. F. Jr., Diabetes 15, 188 (1966)
Haugaard, E. S., and Haugaard, N. J., Biol. Chem., 206, 641 (1954)
Pryor, J., and Berthet, J., Biochim. Biophys. Acta. 43, 556 (1960)
Robinson, B. H. B., and Wright, P. H., J. Physiol. 155, 302 (1961)
Struck, E., Ashmore, J., and Wieland, O., Biochem. Z. 343, 107 (1965)
Williamson, D. H., Mellanby, J., and Krebs, H. A. Biochem. J. 82, 90 (1962)