THE ROLE OF GLUCAGON IN THE REGULATION OF MYOCARDIAL LIPOPROTEIN LIPASE ACTIVITY

J. BORENSZTAJN, P. KEIG AND A.H. RUBENSTEIN

Departments of Pathology and Medicine, Pritzker School of Medicine, The University of Chicago, Chicago, Illinois 60637

Received June 6, 1973

SUMMARY

The role of glucagon in regulating the lipoprotein lipase activities of rat heart and adipose tissue was examined. When starved rats were fed glucose, heart lipoprotein lipase activity decreased while that of adipose tissue increased. Glucagon administration to these animals at the time of glucose feeding prevented the decline in heart lipoprotein lipase activity, but had no effect on the adipose tissue enzyme. When glucagon was administered to fed rats, heart lipoprotein lipase activity increased to levels found in starved animals but there was no change in the adipose tissue enzyme. It is suggested that the reciprocal lipoprotein lipase activities in heart and adipose tissue of fed and starved animals may be regulated by the circulating plasma insulin and glucagon concentrations.

The ability of cardiac muscle and adipose tissue to take up plasma triglyceride fatty acids for oxidation or storage has been well documented (1,2,3). In these tissues, as well as in most extra-hepatic tissues, the uptake of circulating triglyceride fatty acids is regulated by the enzyme lipoprotein (clearing factor) lipase (LPL) (4). In the rat heart and adipose tissue, LPL activity varies according to the nutritional state of the animal. In starved rats the adipose tissue LPL activity is low, while that of the heart is high. When these animals are re-fed, LPL activity in adipose tissue increases and that of the heart declines (5). While there is considerable evidence to indicate that the adipose tissue enzyme is under hormonal control (4), little is known about the factors which regulate myocardial LPL activity.

Recently we have demonstrated (5) that the in vivo increase in LPL activity in the adipose tissue of starved rats re-fed glucose is mediated by insulin. However, the myocardial LPL activity was not directly affected by

this hormone. This conclusion was based on a series of experiments in which the injections of exogenous insulin into control rats and rats made acutely diabetic with mannoheptulose (5) or anti-insulin serum (6) was without effect on the heart LPL activity.

Muller et al. (7) have shown that administration of mannoheptulose and anti-insulin serum to normal animals is accompanied by an increase in circulating glucagon levels. Glucagon levels in serum parallel the changes in myocardial LPL activity being high during periods of starvation and low after feeding (8). These facts prompted us to examine the possibility that glucagon may participate in the in vivo regulation of myocardial LPL activity in the fed and starved state.

METHODS

The conditions for maintenance of the Sprague-Dawley male rats (120-140 g) and the methods used for measurement of serum glucose and LPL activity were similar to those previously described (5), except that the LPL activity in adipose tissue was measured in fresh tissue homogenized in NH_3 - NH_4Cl buffer, pH 8.1. The enzyme activities are expressed in units $\pm$ S.D.: a unit representing 1 μM free fatty acids released/hour incubation/gram wet weight tissue.

Crystalline porcine glucagon was a gift from the Eli Lilly Co. (Indianapolis).

RESULTS

Effect of glucagon administration on heart and adipose tissue LPL activity.

Table 1 shows the results obtained when starved and starved re-fed rats were injected intraperitoneally with glucagon. In 24 hour starved rats, when plasma glucagon levels are known to be elevated (8), administration of the hormone had no effect on the heart LPL activity. When starved animals were re-fed glucose there was a significant ($P < 0.001$) decrease in heart enzyme activity after 3 hours, but this change was prevented if the animals were injected with glucagon.

It is noteworthy that glucagon caused a significant ($P < 0.01$) increment in the adipose tissue enzyme activity of starved animals, albeit not to fed

TABLE 1

Effect of glucagon on heart and adipose tissue LPL activity

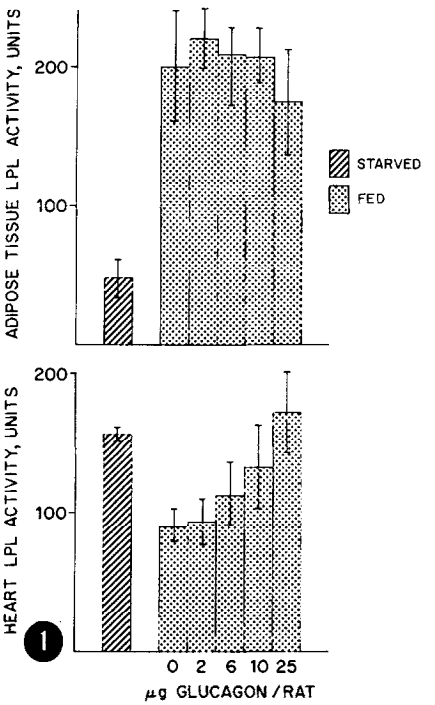
	Injection	Fed	LPL activity, units Heart	LPL activity, units Adipose Tissue	Serum glucose mg/100 ml
1)	Saline	Water	101 ± 10	28 ± 10	68 ± 6
2)	Glucagon	Water	127 ± 10	53 ± 7**	96 ± 12***
3)	Saline	Glucose	70 ± 9	137 ± 12	147 ± 25
4)	Glucagon	Glucose	130 ± 11*	142 ± 7	191 ± 17

Groups of 4 rats that had been starved for 24 hours were injected intraperitoneally with saline or glucagon (25 μg/rat) 3 times at hourly intervals. At the time of the first injection the animals were force fed 3 ml of a glucose solution (60% w/v) or 3 ml of water. All animals were killed 3 hours after the initial injections. *$P < 0.001$ when compared to animals fed glucose and injected with saline. **$P < 0.01$ when compared to animals fed water and injected with saline. ***$P < 0.025$ when compared to animals fed water and injected with saline.

levels. This effect may have been mediated by the rise in plasma insulin which follows glucagon administration (8).

Glucagon dose response on heart and adipose tissue LPL activity. Figure 1 shows the results of increasing doses of glucagon in preventing the decline in heart LPL activity of starved rats re-fed glucose. An injection of 25 μg of glucagon at the same time as glucose feeding was sufficient to completely inhibit the reduction in heart enzyme activity ($P < 0.001$). Glucagon did not affect the marked rise in adipose tissue LPL activity from starved to fed levels.

Effect of glucagon on the heart and adipose tissue LPL activity of fed rats. Figure 2 shows the results obtained when glucagon was administered to rats that had been starved 24 hours and force fed glucose 2 hours prior to the injection of the hormone. In the control animals heart LPL activity was significantly ($P < 0.001$) decreased within 2 hours of glucose feeding and remained at low levels for at least another 2 hours. Within one hour of administering glucagon (50 μg) to the re-fed animals, a significant ($P < 0.001$) increase in



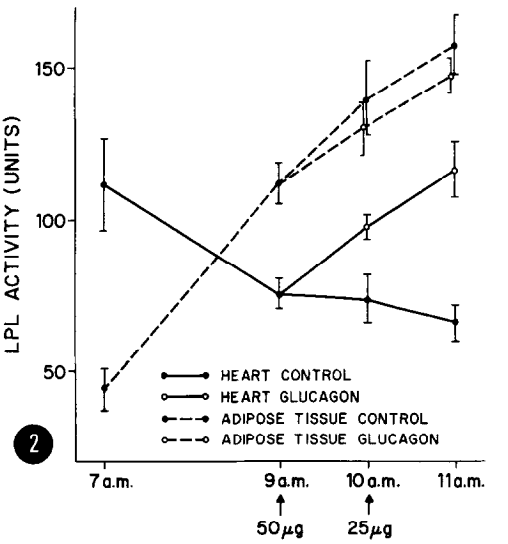


FIGURE 1

Groups of 4 rats that had been starved for 24 hours were force fed 3 ml of a glucose solution (60% w/v) and injected intraperitoneally with different doses of glucagon, as indicated. All animals were sacrificed 3 hours later.

FIGURE 2

Groups of 5 rats that had been starved for 24 hours were force fed 3 ml of a glucose solution (60% w/v) at 7 a.m. Glucagon was injected intraperitoneally into the fed animals as indicated.

myocardial LPL activity was detected. One hour later, following a second injection of glucagon (25 μg), the myocardial enzyme activity reached levels found in the control starved animals. As in the previous experiments, glucagon had no significant effect on the adipose tissue enzyme activity under these conditions.

DISCUSSION

The results of the present investigation indicate that glucagon may participate in the regulation of myocardial LPL activity. This was demonstrated by experiments in which glucagon a) prevented the decline in LPL activity in the heart of starved rats re-fed glucose (Table 1 and Figure 1) and b) increased

LPL activity in the heart of fed rats to values found in starved controls (Figure 2). These findings, together with the observations that adipose tissue LPL activity is regulated by insulin (5), may explain the findings that heart and adipose tissue of fed and starved animals have reciprocal LPL activities. In fed animals, when plasma insulin levels are elevated and glucagon levels are suppressed, adipose tissue enzyme activity is high, while that of the heart is low. In starved animals, on the other hand, when circulating levels of glucagon rise and those of insulin fall, the myocardial enzyme activity increases while that of adipose tissue declines.

We have previously shown (9) that LPL activity in skeletal muscle, like that of the heart, is low in fed rats and high during starvation. Glucagon administration to fed and starved rats produces effects on skeletal muscle LPL activity similar to those observed in the myocardium (unpublished observation). It is therefore possible, that under conditions in which serum glucagon levels are high, the capacity of the heart and skeletal muscle to take up triglyceride fatty acids from the circulation is enhanced. Physical exercise, for example, is associated with an increase in the level of circulating glucagon (10) and a decrease in the concentration of plasma triglycerides (11,12). This latter observation has been interpreted as resulting from the increased utilization of plasma triglyceride fatty acids by the myocardium and exercising muscles (12). Since this uptake of circulating triglyceride fatty acids is facilitated by LPL (4) the rise in plasma glucagon with exercise may be important in regulating this metabolic adaptation. This possibility is supported by the finding that exercised rats have an increased myocardial LPL activity (13).

It should be emphasized that the results of the present investigation do not indicate whether glucagon itself has a direct effect on myocardial LPL or whether its effect is mediated by changes in the concentration of plasma or tissue substrates. Furthermore, glucagon may not be the only hormone involved in the regulation of this enzyme's activity. In other experiments, catecholamines and triiodothyronine (14,15,16) have been shown to affect heart LPL

although in at least one case (17) these findings have not been confirmed.

Acknowledgments - This work was supported in part by the grants PHS-5P17 HL 15062; FDA 72-114; GRS-PHS-RR 05367; AM-13941 and from the Schweppe Foundation, Chicago, Illinois.

REFERENCES

1. Borensztajn, J. and Robinson, D.S., J. Lipid Res. 11, 111, (1970).
2. Enser, M.B., Kunz, F., Borensztajn, J., Opie, L.H. and Robinson, D.S., Biochem. J. 104, 306, (1967).
3. Robinson, D.S., Advan. Lipid Res. 1, 133, (1963).
4. Robinson, D.S., in M. Florkin and E.H. Stotz (Ed), Comprehensive Biochemistry, Amsterdam: Elsevier 18, 51, (1970).
5. Borensztajn, J., Samols, D.R. and Rubenstein, A.H., Am. J. Physiol. 223, 1271, (1972).
6. Borensztajn, J., Keig, P. and Rubenstein, A.H., Unpublished observations.
7. Muller, W.A., Faloona, G.R. and Unger, R.H., J. Clin. Invest. 50, 1992, (1971).
8. Unger, R.H., N. Engl. J. Med. 285, 443, (1971).
9. Borensztajn, J., Otway, S. and Robinson, D.S., J. Lipid Res. 11, 102, (1970).
10. Bottger, I., Schlein, E.M., Faloona, G.R., Knochel, J.P. and Unger, R.H., J. Clin. Endoc. Metab. 35, 117, (1970).
11. Carlson, L.S. and Froberg, S.O., Gerontologia 15, 14, (1969).
12. Holloszy, J.O., Skinner, J.S., Toro, G. and Cureton, T.K., Am. J. Cardiol. 14, 753, (1964).
13. Nikkila, E.A., Torsti, P. and Pentilla, O., Metabolism 12, 863, (1963).
14. Mallov, S. and Cerra, F., J. Pharmacol, Exptl. Therap. 156, 426, (1967).
15. Mallov, S. and Alousi, A.A., Am. J. Physiol. 212, 1158, (1967).
16. Radomski, M.W. and Orme, R., Am. J. Physiol. 220, 1852, (1971).
17. Nikkila, E.A., Torsti, P. and Pentilla, O., Life Sci. 4, 27, (1965).