Vol. 91, No. 1, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 14, 1979 Pages 227-232

ROLE OF LIPOPROTEIN LIPASE IN REGULATING ENDOGENOUS TRIACYLGLYCEROLS IN RAT HEART

L. B. Oscai

Department of Physical Education, University of Illinois at Chicago Circle, Chicago, Illinois 60680

Received September 14, 1979

Summary - Administration of glucagon (10 µg/rat) to the intact animal increased the levels of lipoprotein lipase activity by 92% in the heparin-non-releasable fraction of the heart. At the same time, cardiac levels of triacylglycerols were reduced 47% and free fatty acids were increased about 2-fold. In contrast, the administration of a lower dose of glucagon (0.5 µg/rat) resulted in an 80% reduction in lipoprotein lipase activity in the heparin perfused myocardium. At the same time, triacylglycerols were increased 44% and free fatty acids were decreased 69%. These results provide circumstantial evidence that lipoprotein lipase is involved in the regulation of endogenous triacylglycerols such that higher levels of enzyme activity result in cardiac lipolysis and, conversely, lower levels result in triacylglycerol production.

Lipoprotein lipase (LPL) in the heart exists in two distinct fractions: a heparin-releasable fraction (capillary bound) and a heparin-non-releasable fraction (tissue bound) (1,2). In the course of studies attempting to provide an explanation for a rise in myocardial free fatty acids (FFA) seen 24 h after exercise in previously untrained rats (3), it was noted that both LPL activity and FFA were elevated in the heparin-non-releasable fraction. These results raised the possibility that LPL, in addition to its well documented role as a "clearing-factor lipase" responsible for the hydrolysis of plasma triacylglycerols (1,2,4-8), might function as a mobilizing enzyme capable of hydrolyzing endogenous triacylglycerols in the heart. In the present study, this possibility was tested using glucagon, instead of exercise, as the inducing stimulus. Although preliminary studies showed that both agents were capable of elevating LPL activity in the heart, the magnitude of the increase seen with hormone (10 µg/rat) was much greater than that seen with exercise. Nevertheless, the results obtained led us to test a second possibility that LPL might be involved in the regulation of endogenous triacylglycerols in rat heart.

0006-291X/79/210227-06$01.00/0

METHODS

Animal care - Male rats of a Wistar strain (Charles River), weighing approximately 200 g, were used. They were provided unrestricted access to a diet of Purina rat chow and water. The animal room was maintained at a temperature between 21 and 23°C and lighted between 7:30 a.m. and 7:30 p.m. Rats were sacrificed between 9:00 a.m. and 9:30 a.m. after an overnight fast.

Tissue preparation and assay methods - To measure heparin-non-releasable LPL activity, the heparin perfused heart was homogenized in 0.025 N NH_3-NH_4Cl buffer at pH 8.1 using a Duall ground glass grinder (Kontes Glass Co., Evanston, IL.). The tissue concentration of the homogenates was 50 mg/ml. The composition of the assay medium and the procedures for incubation, extraction, and measurement of unesterified FFA released into the assay medium have been described (12). Lipolytic activities of the hearts had the characteristics of LPL, e.g., the reaction was inhibited in the absence of serum and in the presence of 1 M NaCl. Enzyme activities are expressed as units ± SE, 1 unit representing 1 µmol of unesterified fatty acids released into the assay medium/h of incubation.

Triacylglycerols were extracted from the heart, that had been carefully cleaned free of any adherent adipose tissue and connective tissue, with alcohol-ether 3:1 (v/v) according to the method of Entenman (13). The concentration of triacylglycerols was determined as described by Fletcher (14). FFA were measured using the method of Trout et al. (15). The buffer used and the tissue concentration of the homogenates were the same as that used for the assay of LPL.

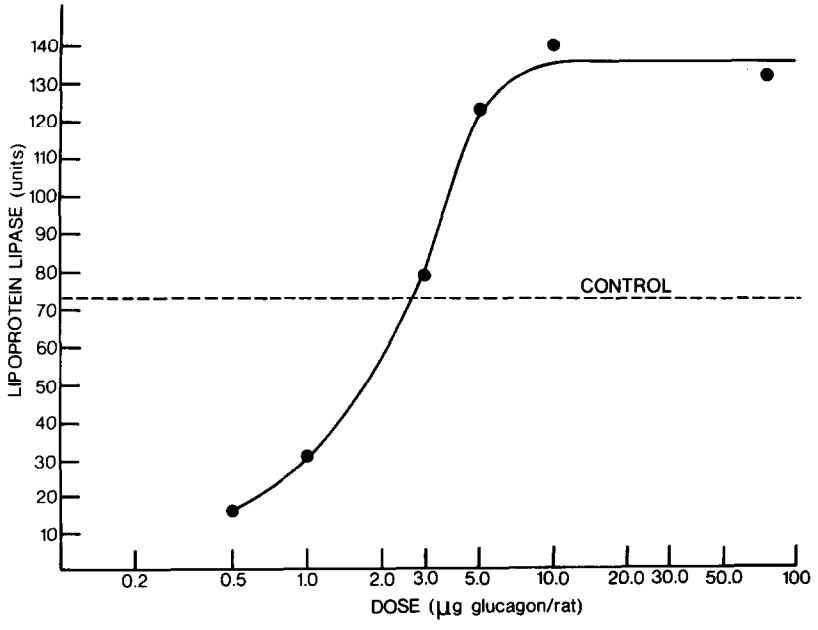

Figure 1. Rats were injected with glucagon; control rats were injected with 0.9% NaCl. Information regarding glucose feeding, and procedures for isolation and perfusion of the heart are given under "Table 1". Assays for lipoprotein lipase activity were carried out on heparin perfused tissue as described under "Methods". Each point is the mean for hearts from 9 rats.

RESULTS

Heparin-non-releasable LPL activity - An intraperitoneal injection of glucagon (10 μg/rat) increased LPL activity from 73 ± 0.8 units to 140 ± 1 units measured in the heparin-non-releasable fraction of the heart (Figure 1; $P<0.001$). In contrast, a lower dose of hormone (0.5 μg/rat) decreased LPL activity from 73 ± 0.8 units to 15 ± 0.5 units (Figure 1; $P<0.001$). To determine if enzyme activity was proportional to concentration of inducing stimulus, rats were injected with 0.5 μg/rat or 1 μg/rat of glucagon. Figure 1 shows that heparin-non-releasable LPL activity increased from 15 ± 0.5 units to 31 ± 0.9 units. Injections of glucagon of 3 μg/rat, 5 μg/rat, and 75 μg/rat yielded values of LPL activity of 79 ± 0.3 units, 123 ± 2 units, and 132 ± 1 units, respectively (Figure 1).

Triacylglycerol and FFA - Table 1 shows that intraperitoneal injection of glucagon (10 μg/rat) resulted in a 47% reduction in the concentration of triacylglycerols of the myocardium. At the same time, FFA increased approximately 2-fold. A lower dose of glucagon (0.5 μg/rat), on the other hand, resulted in a significant rise in the triacylglycerol content of the heart (Table 2). FFA under these conditions decreased 69% (Table 2). When the dose of glucagon was increased from 0.5 μg/rat to 1 μg/rat, tissue triacylglycerols decreased from 3.69 ± 0.10 μmol/g tissue to 3.12 ± 0.10 μmol/g tissue ($P<0.01$).

DISCUSSION

The existence of a "clearing-factor lipase" in tissue with properties of the enzyme present in post-heparin plasma was first described about 25 years ago by Korn (16,17). In the intervening years, the concept has developed that LPL functions exclusively as a "clearing-factor lipase" responsible for the hydrolysis of triacylglycerols from plasma chylomicrons and very low density lipoproteins (1,2,4-8). Presumably, LPL is formed or activated within tissue cells (1,2,18-22) and then transported to the lumenal surface of endothelial cells of capillaries (23,24), the site of plasma triacylglycerol hydrolysis (1,2,4-8). In the heart, it is possible to distinguish LPL bound to the cap-

TABLE 1

Triacylglycerols and free fatty acids in the heart after glucagon treatment to the intact rat

Injection	Triacylglycerol	Free fatty acid
	μmol/g tissue	
Saline	2.57 ± 0.08	4.32 ± 0.12
Glucagon	1.36 ± 0.08*	9.00 ± 0.13*

Values are means ± SE for hearts from 9 rats. *Glucagon versus saline treated, $P < 0.001$.

Rats were injected intraperitoneally with glucagon (10 μg/rat). Control rats were injected with an equivalent volume of 0.9% NaCl. At the same time, animals were force fed 3 ml of a glucose solution (60% w/v). Glucose was used to stimulate insulin release which has been shown to have a powerful anti-lipolytic effect on the hormone-sensitive lipase system of adipose tissue (9) even though such a lipase system has never been identified in the heart (10). It has been shown that ingested glucose has no effect on myocardial lipoprotein lipase activity in glucagon treated rats (11). Thirty min later, hearts were isolated and perfused for 120 s at a rate of 3 ml/30 s with Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5% (v/v) rat serum and 5 units heparin/ml. The medium [continually gassed with O_2 + CO_2 95:5 (v/v)] was drawn from a reservoir, then perfused through the heart in a nonrecirculatory system by a Gilson minipuls 2 peristaltic pump. At the end of the 2 min perfusion period, perfusates were triacylglycerol, free fatty acid, and lipoprotein lipase free. Assays for triacylglycerols and free fatty acids were carried out as described under "Methods".

TABLE 2

Triacylglycerols and free fatty acids in the heart after glucagon treatment to the intact rat

Injection	Triacylglycerol	Free fatty acid
	μmol/g tissue	
Saline	2.57 ± 0.08	4.32 ± 0.12
Glucagon	3.69 ± 0.10*	1.35 ± 0.06*

Values are means ± SE for hearts from 9 rats. *Glucagon versus saline treated, $P < 0.001$.

Rats were injected intraperitoneally with glucagon (0.5 μg/rat). Controls were injected with an equivalent volume of 0.9% NaCl. Information regarding glucose feeding, and procedures for isolation and perfusion of the heart are given under "Table 1". Assays for triacylglycerols and free fatty acids were carried out on heparin perfused tissue as described under "Methods".

illary walls from that bound to tissue by the former's property of being releasable from tissue by action of heparin (1,2).

In the present study, treatment with glucagon (10 µg/rat) resulted in a marked rise in LPL measured in the heparin-non-releasable fraction of the heart. The rise in enzyme activity was accompanied by a reduction in the concentration of cardiac triacylglycerols and a marked increase in FFA. These results provide suggestive evidence that LPL functions as a mobilizing enzyme capable of hydrolyzing endogenous triacylglycerols. The administration of a lower dose of glucagon (0.5 µg/rat), on the other hand, markedly lowered LPL activity in the heparin-non-releasable fraction of the heart. The lower levels of enzyme activity were accompanied by elevated levels of triacylglycerols and lower levels of tissue FFA. These results provide circumstantial evidence that LPL is involved in the regulation of endogenous triacylglycerols in the heart. It is involved such that lower levels of LPL activity result in the endogenous production of triacylglycerols and, conversely, higher levels of enzyme activity result in cardiac lipolysis.

As mentioned earlier, lipolytic activities of the heparin perfused tissue had the characteristics of LPL. For example, triacylglycerol lipolysis was inhibited in the absence of serum. Therefore, if LPL is capable of hydrolyzing intramuscular triacylglycerols, as our results seem to indicate, then tissue cells must contain a protein necessary for lipolytic activity similar to that found in the serum (17).

It should be mentioned that Borensztajn et al. (11) reported that glucagon (75 µg/rat) administered at intervals over a 3 h long period elevated LPL activity measured in whole homogenates of the heart. Results of the present study support this finding and extend it to show that glucagon at a lower dose (0.5 µg/rat) markedly reduces enzyme activity. Taken together, the results provide evidence that glucagon participates in the regulation of LPL in the rat heart.

ACKNOWLEDGMENTS

The technical assistance of Roseann Caruso and Ann Wergeles is gratefully acknowledged. This work was supported by Public Health Service Research Grants AM-17357, K04-00216, and HD-10987.

REFERENCES

1. Robinson, D.S. (1970) Compr. Biochem., 18, 51-116.
2. Borensztajn, J., and Robinson, D.S. (1970) J. Lipid Res., 11, 111-117.
3. Oscai, L.B. (1979) Can. J. Physiol. Pharmacol., 57, 485-489.
4. Garfinkel, A.S., Baker, N., and Schotz, M.C. (1967) J. Lipid Res., 8, 274-280.
5. Scow, R.O., Mendelson, C.R., Zinder, O., Hamosh, M., and Blanchette-Mackie, E.J. (1973) in Dietary Lipids and Postnatal Development (Galli, C., Jacini, G., and Pecile, A., eds) pp. 91-114, Raven Press, New York.
6. Hamosh, M., and Hamosh, P. (1975) Biochim. Biophys. Acta, 380, 132-140.
7. Chajek, T., and Eisenberg, S. (1978) J. Clin. Invest., 61, 1654-1665.
8. Higgins, J.M., and Fielding, C.J. (1975) Biochemistry, 14, 2288-2292.
9. Kono, T., Robinson, F.W., and Sarver, J.A. (1975) J. Biol. Chem., 250, 7826-7835.
10. Lech, J.J., Jesmok, G.J., and Calvert, D.N. (1977) Fed. Proc., 36, 2000-2008.
11. Borensztajn, J., Keig, P., and Rubenstein, A.H. (1973) Biochem. Biophys. Res. Commun., 53, 603-608.
12. Borensztajn, J., Samols, D.R., and Rubenstein, A.H. (1972) Am. J. Physiol., 223, 1271-1275.
13. Entenman, C. (1957) Methods Enzymol., 3, 301-302.
14. Fletcher, M.J. (1968) Clin. Chim. Acta, 22, 393-397.
15. Trout, D.L., Estes, E.H., Jr., and Friedberg, S.J. (1960) J. Lipid Res., 1, 199-202.
16. Korn, E.D. (1955) J. Biol. Chem., 215, 1-14.
17. Korn, E.D. (1955) J. Biol. Chem., 215, 15-26.
18. Chajek, T., Stein, O., and Stein, Y. (1975) Biochim. Biophys. Acta, 380, 127-131.
19. Davies, P, Cryer, A., and Robinson, D.S. (1974) FEBS Letters, 45, 271-275.
20. Schotz, M.C., and Garfinkel, A.S. (1972) Biochim. Biophys. Acta, 270, 472-478.
21. Stewart, J.E., and Schotz, M.C. (1971) J. Biol. Chem., 246, 5749-5753.
22. Stewart, J.E., and Schotz, M.C. (1974) J. Biol. Chem., 249, 904-907.
23. Borensztajn, J., Rone, M.S., and Sandros, T. (1975) Biochim. Biophys. Acta, 398, 394-400.
24. Chajek, T., Stein, O., and Stein, Y. (1975) Biochim. Biophys. Acta, 388, 260-267.