PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 162, 389–391 (1979)

Quantitative Aspects of Lipoprotein Lipase Release by Heparin in Mice (40688)[1]

S. B. GERTNER AND S. SHERR

Departments of Pharmacology and Biochemistry, New Jersey Medical School—CMDNJ, 100 Bergen St., Newark, New Jersey 07103

Although heparin has been known to release lipoprotein lipase (diacylglycerol acylhydrolase, EC 3.1.1.34) since 1943 (1), the quantitative aspects related to dosage have never been fully determined. Furthermore, only the data of Robinson *et al.* (2) are available concerning the half-life of enzyme action, once released into the blood. Olsson *et al.* (3) and Estes *et al.* (4) published data on the volume of distribution of heparin and kinetic data on heparin's anticoagulant action in man. More recently, McAvoy (5) determined the biologic half-life of heparin. In this paper, we present quantitative dose–response data on the release of lipoprotein lipase by heparin in mice, as well as information on time-duration action of released lipoprotein lipase in serum.

Materials and Methods. Seventy male Swiss–Webster mice (weighing 20–30 g) were used in this investigation. Heparin sodium was injected intravenously in the tail vein in a volume of 0.1 ml. Axillary blood was obtained from etherized mice, allowed to clot, and then spun down in a refrigerated centrifuge at 2500 rpm for 30 min at 5°. (In previous experiments we found that etherization did not affect lipoprotein lipase release.) The clear serum was either tested immediately or frozen at −10° and used on subsequent days. The enzyme was found to be quite stable and showed no discernible diminution in activity on storage at −10° for 20 days. It was always tested either immediately or within a few days after collection.

Lipoprotein lipase assay. To determine the level of lipoprotein lipase, 0.6 ml of serum was added to a flask in which 5.4 ml of [^{14}C]triolein substrate (see below) was placed; it was incubated in a shaking incubator for 20 min at 27° (according to a modification of the method of Schotz (6, 7)). At intervals of 1.5, 10, and 20 min, 0.5-ml samples were removed from the flask and added to isopropanol–sulfuric acid (40:1) solution to stop the reaction. Then 5 ml of hexane and 1 ml of water were added to each aliquot and the tubes were shaken for 1 min in a mechanical shaker. The upper layer containing the hexane phase was separated and shaken with 2 ml of 0.1 *N* KOH for 10 min. The lower phase contains the potassium oleate generated by enzymic action on the triolein substrate. The 1-ml aliquots of the lower phase were added to 10 ml Aquasol and counted in an Intertechnique liquid scintillation spectrometer, Model SL-30. All results were corrected to dpm and converted to nmol oleic acid generated/min.

Triolein substrate for enzyme assay. The 3 ml of ^{14}COOH-triolein (New England Nuclear Corp, 55 mC/m*M*) in hexane solution and containing 15 μCi of radioactivity was evaporated to dryness under nitrogen and 270 mg triolein (fatty acid free), was added to the beaker. Fatty acid-free albumin (600 mg) was added and 1.8 ml Triton X-100 (1%) and 34.2 ml Tris buffer (0.2 *M*, pH 8.0). The mixture was sonicated in an ice bath for 5 min at full output of a Bronson sonicator. The emulsion was stable for at least 30 min. If not used within 30 min, the emulsion was resonicated before use. The emulsion could be stored by freezing at −10°, thawed, and resonicated before use.

All results are expressed as the mean ± SE. Triolein was 99% pure and obtained from Sigma Chemical Company as well as fatty acid-free albumin (from fraction V). Triolein-^{14}COOH was obtained from New England Nuclear and has a specific activity of 55 mCi/mmol. Heparin sodium was made from porcine intestinal mucosa and was obtained from Sigma Chemical Company. It was assayed by the company at 169.9 U.S.P. units/mg.

Results. Control studies in fasted animals in which saline was injected intravenously, showed extremely low levels of nonspecific

[1] Supported by NIH Grant HL 18607.

0037-9727/79/110389-03$01.00/0

lipase activity that generated less than 0.01 nmol oleic acid/min. This amount of enzyme activity was negligible compared to the quantity of oleic acid generated when heparin was given. Control substrates without enzyme showed a small degree of hydrolysis on incubation and produced the equivalent of 0.5 nmol oleic acid. This value was subtracted from the results reported and was consistent in all experiments.

To determine levels of serum lipoprotein lipase, groups of five mice were injected with heparin, i.v., at six dosage levels of 0.1, 0.5, 1.0, 4.0, 8.0, and 12.0 units/g body wt. The animals were bled 8 min after the injection and the serum prepared as indicated under Methods.

The results of this study show that with doses of 0.1 unit/g there are small but measureable levels of lipoprotein lipase released into the blood (Fig. 1). With increasing dosage of heparin above 0.1 unit/g, there was a consistent and proportional increase in the amount of lipoprotein lipase released into the blood. The quantity of enzyme released into the blood appeared to reach a plateau maximum at a dosage of heparin of 4 units/g. The form of the dose–response curve for the release of the enzyme into blood was sigmoidal and typical of classical dose–response curves. It is shown in Fig. 1.

In additional experiments in five mice, a dose of heparin of 0.05 units/g, in some animals caused the release of lipoprotein lipase activity into the blood and in others was almost without effect. These actions were not studied further because of great variability and therefore are not included in the graph shown in Fig. 1. This dose is probably threshold in a number of mice.

Following these experiments, we studied the time-duration characteristics of released lipoprotein lipase in blood. We employed five groups of seven mice and injected each mouse with 1 unit/g heparin, i.v. Blood was obtained from these mice at 30 sec, and 1, 5, 15, and 60 min following the injections. The sera were prepared as indicated before, and the levels of lipoprotein lipase were determined.

The data (Fig. 2) showed that heparin released lipoprotein lipase rapidly on entering the vascular system. The earliest practical time for measurement was 30 sec after the administration of heparin. Such measurements were extremely difficult to do accurately and showed very high levels of the enzyme in serum. However, due to very high errors in sampling, these data are not included in Fig. 2.

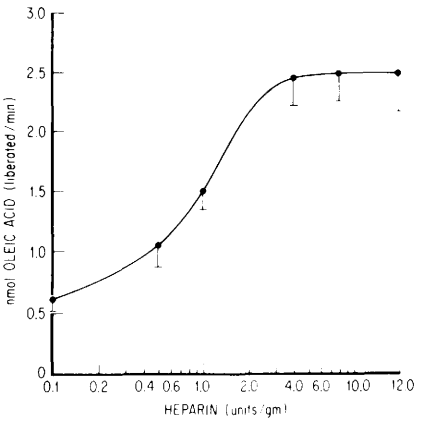

FIG. 1. Curve of levels of lipoprotein lipase present in serum 8 min following the intravenous injections of graded doses of heparin. Activity of lipoprotein lipase is given as nmol oleic acid liberated/min by enzymic action on [^{14}C]triolein substrate (see text).

Once released into the blood, there was an extraordinary rapid decay of lipoprotein lipase levels in serum. Graphical estimation of the half-life, showed a half-life of lipoprotein lipase in serum of approximately 4 min. As indicated in Fig. 2, at 1 min after injection, serum lipoprotein lipase was at the highest level in these experiments and showed activity equivalent to 3.8 nmol oleic acid liberated/min. At 5 min following injection the enzyme activity in the serum had dropped to 1.8 n*M* oleic acid liberated/min. Sixty minutes after injection the serum lipoprotein lipase levels had returned almost to control values. Three hours after injection there was no discernible activity in the serum. Thus, it appeared from these studies that the total effective period for lipoprotein lipase activity in the serum, once released by heparin (1 unit/g), was at most 1 hr following injection.

To obtain some preliminary understanding of the disposition of serum lipoprotein lipase once released into the blood, mouse liver homogenate in isotonic saline was prepared and incubated in duplicate with serum (0.5 ml) at 37° containing known levels of the enzyme. Thirty minutes after incubation samples were taken and measured for lipoprotein lipase activity. After correcting for dilution,

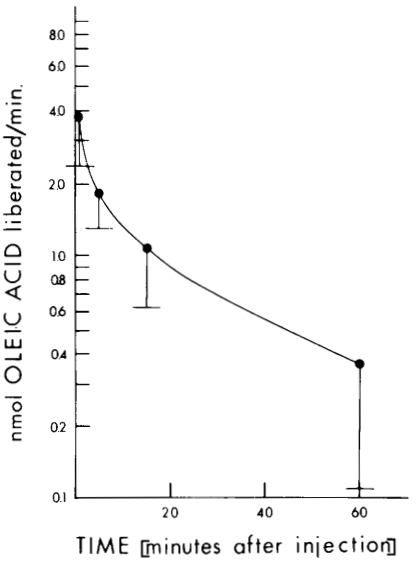

FIG. 2. Disappearance of lipoprotein lipase activity from serum following its release by the intravenous injections of 1 unit/g heparin at zero time. Activity of the enzyme is given as nmol oleic acid liberated/min by enzymic action on [^{14}C]triolein substrate (see text).

the results of two experiments showed that about 40% of the lipoprotein lipase activity present in the original serum disappeared over 30 min.

Discussion. These data show that the release of lipoprotein lipase in mice follows a classical sigmoidal dose–response curve. Furthermore, the effective range of this dose–response curve is from 0.1 to 12 units/g of heparin. It is evident from the curve generated that no greater increase in lipoprotein lipase activity occurred with doses higher than 4 units/g heparin. Even though heparin has been known to release lipoprotein lipase for many years, exact dose–response data have been lacking. Other than the report of Robinson and Harris in man, there have not been quantitative data of this kind.

The results on the half-life of free lipoprotein lipase in serum were similar in some respects to man, where a longer half-life of 9 min has been reported by Robinson and Harris. There is no doubt that in mice, once the enzyme is released from its storage sites, it undergoes rapid disappearance from the blood. Our preliminary results with liver homogenate reveal only that liver can inactivate the enzyme molecule. More sophisticated experiments would have to be done to determine whether this is outright destruction of the enzyme or merely complexing with charged anionic compounds.

Although, *in vivo*, in the clearing of chylomicrons from blood, there is evidence that lipoprotein lipase remains attached to the capillary endothelium (8), once the enzyme is released free into the circulating blood by heparin treatment, enzyme activity diminishes rapidly. This suggests that the functional groups necessary for lipolytic activity are very quickly blocked or destroyed.

In man, lipoprotein lipase once released has a comparable short half-life to that observed in mice in these experiments. Unless there is careful consideration of the time interval following heparin injection, erroneous interpretations will result concerning the levels of serum lipoprotein lipase. It can be seen from Fig. 2, that had we chosen 1 min following injection for our dose–response curve in Fig. 1, the levels of lipoprotein lipase would have been almost threefold higher.

There have been no studies to date, to indicate how rapidly lipoprotein lipase is synthesized to replace that which is released by heparin or other polyanionic drugs. It is unlikely to be related to the half-life reported for heparin (4, 5). Such data would be extremely valuable for determining the kinetics and turnover rate of this enzyme, particularly because so many individuals are being treated with heparin.

We wish to thank Paul Segal and Martin Ontell for their technical help in this project.

1. Hahn, P. F., Science **98,** 19 (1943).
2. Robinson, D. S., and Harris, P. M., Quart. J. Exp. Physiol. **44,** 80 (1959).
3. Olsson, P., Lagergren, H., and Ek, S., Acta Med. Scand. **173,** 619 (1963).
4. Estes, J. W., Pelikan, E. W., and Kruger-Thiemer, E., Clin. Pharmacol. **10,** 329 (1969).
5. McAvoy, T. J., Clin. Pharmacol. Ther. **25,** 372 (1969).
6. Schotz, M. C., Garfinkel, A. S., Huebotter, F. R., and Stewart, J. E., J. Lipid Res. **11,** 68 (1970).
7. Sherr, S., and Gertner, S., Exp. Mol. Pathol. **25,** 60 (1976).
8. Olivecrona, T., Bengtsson, G., Marklund, S., Lindahl, U., and Hook, M., Fed. Proc. **36,** 60 (1977).

Received June 28, 1978. P.S.E.B.M. 1979, Vol. 162.