Activation of Lipoprotein Lipase
COMPARATIVE STUDY OF MAN AND OTHER MAMMALS

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ABSTRACT
Injection of heparin into a number of animal species releases lipoprotein lipase into the circulation. We have studied the effect of heparin added in vitro to the lipase activity in post-heparin serum from six mammalian species. With the exception of man, all animals were studied under anesthesia. Our results demonstrate that only post-heparin serum from the rat developed increasing lipoprotein lipase activity when increasing concentrations of heparin were added to the assay system. Heparin decreased activity in the other species. These results prompted us to test the effect of adding rat serum to post-heparin serum from the other species in the presence of increasing concentrations of heparin. Rat serum stimulated lipoprotein lipase activity markedly. In guinea pigs, post-heparin serum activity increased 2,700% at a heparin concentration of 1.0 U/ml in the assay system. This effect may be related to the extremely low level of high-density lipoprotein in the guinea pig and the presence of a unique high-density lipoprotein in the rat.

ADDITIONAL KEY WORDS post-heparin serum rat dog guinea pig coypu rabbit heparin lipoproteins triglycerides enzyme activation

Intravenous injection of heparin into the circulation causes clearing of lipemic plasma by hydrolysis of circulating triglycerides (1). The enzyme system responsible for the clearing reaction was characterized in heart muscle by Korn in 1955 and was designated as a heparin-activated lipoprotein lipase (LPL) (2). He found that LPL would hydrolyze a triglyceride emulsion to free fatty acids (FFA) and free glycerol only if the emulsion had been activated with serum. His results suggested that the most probable activator in serum was an \( \alpha \)-lipoprotein and that heparin probably functioned as a cofactor or prosthetic group for the enzyme (3). However, Korn also observed an inhibition of LPL activity by a high concentration of in-vitro heparin (2). Brown, Boyle and Anfinsen found a logarithmic increase in the percent inhibition of clearing activity as the in-vitro heparin concentration was increased in post-heparin serum from dogs (4). In contrast to the observation on the effects of in-vitro heparin on LPL activity from dog serum, Naito and Felts observed that addition of heparin in vitro to post-heparin serum from rats resulted in a marked increase in LPL activity (5).

We studied post-heparin serum obtained from animals representing six mammalian species: rat, guinea pig, man, coypu, rabbit, and dog. In each, the effect of heparin added in vitro to post-heparin serum was studied. We observed distinct species differences in LPL activity when heparin was added to the assay system. In addition, we also studied the effect of adding rat serum to the assay system. This resulted not only in changes in LPL activity but also altered the manner in which...
the post-heparin serum activity responded to heparin addition in vitro.

Methods

Six species of mammals were studied in six separate experiments. All animals had free access to food and water until the time of the experiment. In experiment 1, male Long Evans rats weighing 325 to 375 g were anesthetized by ether inhalation. Midline laparotomies were performed on three series of 10 rats. The inferior vena cava and aorta were exposed. Ten ml of blood was collected from the aortas of five rats and pooled. Heparin in a dose of 10 U/kg was then injected into five other rats via the inferior vena cava, and 10 ml of blood was collected from the abdominal aorta of each rat for 2 to 3 minutes following the injection and pooled.

In experiment 2, midline laparotomies were performed under ether anesthesia on three series of 10 male guinea pigs of the English variety weighing 600 to 600 g. The inferior vena cava and aorta were exposed. Twenty ml of blood was collected from the aortas of five guinea pigs and pooled. Heparin in a dose of 20 U/kg was then injected into five other guinea pigs via the inferior vena cava and 10 to 20 ml of blood was collected from the abdominal aorta of each for 2 to 3 minutes following injection and pooled.

In experiment 3, three human volunteers weighing 70 to 80 kg received heparin in a dose of 20 U/kg intravenously into an antecubital vein after 50 ml of blood was withdrawn. Fifty ml of blood was then collected from the opposite antecubital vein for 3 to 4 minutes following injection of heparin.

In experiment 4, two male coypus weighing 3.5 and 7.4 kg were studied separately. The coypus were anesthetized with pentobarbital (Diabutal, Diamond Laboratories Inc., Des Moines, Iowa) and 7.4 kg were studied separately. The coypus were anesthetized with pentobarbital (Diabutal, Diamond Laboratories Inc., Des Moines, Iowa) and 7.4 kg were studied separately. The coypus were anesthetized with pentobarbital (Diabutal, Diamond Laboratories Inc., Des Moines, Iowa) and 7.4 kg were studied separately. The coypus were anesthetized with pentobarbital (Diabutal, Diamond Laboratories Inc., Des Moines, Iowa) and 7.4 kg were studied separately. The coypus were anesthetized with pentobarbital (Diabutal, Diamond Laboratories Inc., Des Moines, Iowa) and 7.4 kg were studied separately. The coypus were anesthetized with pentobarbital (Diabutal, Diamond Laboratories Inc., Des Moines, Iowa) and 7.4 kg were studied separately. The coypus were anesthetized with pentobarbital (Diabutal, Diamond Laboratories Inc., Des Moines, Iowa) and 7.4 kg were studied separately. The coypus were anesthetized with pentobarbital (Diabutal, Diamond Laboratories Inc., Des Moines, Iowa) and 7.4 kg were studied separately. The coypus were anesthetized with pentobarbital (Diabutal, Diamond Laboratories Inc., Des Moines, Iowa) and 7.4 kg were studied separately. The coypus were anesthetized with pentobarbital (Diabutal, Diamond Laboratories Inc., Des Moines, Iowa) and 7.4 kg were studied separately. The coypus were anesthetized with pentobarbital (Diabutal, Diamond Laboratories Inc., Des Moines, Iowa) and 7.4 kg were studied separately.

In experiment 5, three male New Zealand white rabbits weighing 2.5 to 3.5 kg were given spinal anesthesia using 2% lidocaine with epinephrine (Astra Pharmaceutical Products Inc., Worcester, Mass.). The rabbits were studied separately. A size P.E. 160 polyethylene catheter was placed in a carotid artery of each dog and 50 ml of blood was collected. Heparin in a dose of 20 U/kg was then injected intra-arterially. For 3 to 4 minutes following injection, 50 ml of blood was collected from the arterial catheter.

In experiment 6, three male mongrel dogs weighing 9 to 27 kg were anesthetized with pentobarbital administered intravenously; the dogs were studied separately. A silicone rubber catheter (Silastic, i.d. 0.062 inch, o.d. 0.125 inch, Dow Corning Corp., Midland, Mich.) was inserted into a carotid artery of each dog and 50 ml of blood was collected. Heparin in a dose of 20 U/kg was then injected intra-arterially and 50 ml of blood was withdrawn from the catheters for 3 to 4 minutes following the injection.

The blood samples taken before and after heparin were placed in glass beakers and defibrinated immediately by stirring with wooden sticks. The defibrinated blood was then centrifuged at 1,000 g to remove the red cells and the serum was placed on ice during the period prior to analysis of LPL activity.

Lipase activity was assayed in triplicate by a modification of Robinson's method (6). The assay system consisted of the following: 1.50 ml of triglyceride substrate consisting of one part Intralipid (A.B. Vitrum, Stockholm, Sweden) and one part 0.15M NaCl that had been "activated" by incubation for 30 minutes at 37°C with 8 parts of fresh serum obtained from the species to be tested; 0.75 ml of 1.35M tris buffer, pH 8.4; 2.25 ml of a 15% (w/v) solution of bovine albumin (Pentex Inc., Kankakee, Ill.), pH 8.4; 1.0 ml of 0.025M N74OH adjusted to pH 8.6 with HCl; 1.0 ml of test serum; and 0.5 ml of 0.15M NaCl or 0.1 ml of a heparin solution in 0.15M NaCl and 0.4 ml of 0.15M NaCl or 0.4 ml of rat serum. Incubations of this assay system were carried out at 37°C for 1 hour. Released FFA were extracted by the method of Dole, as modified by Trout, Estes and Frieberg (7). The FFA were then titrated by a modification of the method of Salaman and Robinson (8). One unit of LPL activity is equivalent to 1 µmole of FFA released/ml of serum/hour. Titrations were made on aliquots removed at 0, 30, and 60 minutes or at 0 and 60 minutes from the assay system. In earlier experiments on post-heparin serum from rat, dog, and rabbit the rate of release of FFA was constant over a 60-minute period.

In all experiments the effect of heparin added to the assay system was studied. The following additions were made before incubation: no heparin; heparin to give a final concentration of 0.01, 0.1, 1.0, and 10 U/ml of the incubation mixture. The effect of rat serum (and human serum in experiment 2) on the lipase reaction was also studied in the assay system. Increasing concentrations of heparin were added to this mixture as described above.

Results

Experiment 1.—The in vitro effect of in-
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Figure 1
Effect of adding heparin in vitro to the assay system on rat post-heparin lipoprotein lipase activity. 100% = 1.39 units of lipase activity. In this and all other illustrations PHLA = post-heparin lipoprotein lipase activity, and PHS = post-heparin serum.

Increasing concentrations of heparin on the LPL activity of post-heparin serum obtained from the rat was studied. With three series of rats, the post-heparin serum had a mean activity of 1.39 LPL units with a range of 0.99 to 1.93. When heparin was added to the assay system in increasing concentrations, a marked increase in LPL activity resulted (Fig. 1). A peak of LPL activity was reached with a heparin concentration of 1.0 U/ml. The mean of the maximum increase was 714% with a range of 499 to 995%.

Experiment 2.—Guinea pig post-heparin serum had a mean activity of 0.55 LPL units with a range of 0.45 to 0.65. The addition of heparin to the assay system did not elicit any significant change in activity. However, when rat serum was added to the assay system without adding heparin there was a 1,200% increase in LPL activity. When heparin was then added, LPL activity increased to a maximum of 2,700% at a concentration of 1.0 U/ml and decreased only slightly at 10 U/ml. When human instead of rat serum was added to the assay system there was a 1,400% increase in LPL activity when no heparin was added and a maximum increase of 1,700% when heparin was added to a concentration of 0.1 U/ml. The LPL activity then decreased markedly as the heparin concentration was increased further (Fig. 2).

Experiment 3.—Human post-heparin serum had a mean activity of 3.88 LPL units with a range of 1.87 to 6.38. The addition of heparin to the assay system resulted in a depression of the post-heparin serum LPL activity as the heparin concentration was increased. When rat serum was added to the assay system, LPL activity increased as the in-vitro heparin was increased, and a peak LPL activity was reached at a concentration of 1.0 U/ml (Fig. 3).

Experiment 4.—Coypu post-heparin serum had a mean activity of 2.63 LPL units with a range of 1.95 to 3.30. The addition of heparin to the assay system resulted in a depression of the post-heparin serum LPL activity as the
Experiment 5.—Rabbit post-heparin serum had a mean activity of 0.86 LPL units with a range of 0.69 to 1.14. The addition of heparin to the assay system resulted in a depression of the post-heparin serum LPL activity as the heparin concentration was increased. When rat serum was added to the assay system, LPL activity was increased, and as the heparin concentration was increased, a peak LPL activity was reached at a concentration of 10 U/ml (Fig. 5).

Experiment 6.—Dog post-heparin serum had a mean activity of 3.13 LPL units with a range of 2.87 to 3.60. The addition of heparin to the assay system resulted in a depression of the post-heparin serum LPL activity as the heparin concentration was increased. When rat serum was added to the assay system, LPL activity increased, and as the heparin was increased, a peak LPL activity was reached at a concentration of 1.0 U/ml (Fig. 6).
Discussion

The postulated function of heparin as a prosthetic group for LPL, which aids in the formation of an enzyme-substrate complex with an activated triglyceride emulsion, follows from studies of the tissue enzyme (2, 9, 10). The interaction of the enzyme with the surface of the activated substrate is undoubtedly dependent on the nature of the activation factor, which could vary from species to species. In addition, the effects of heparin on the apoenzyme in increasing its affinity for the activated substrate may depend on the nature of the apoenzyme, which may vary from species to species.

The highest levels of post-heparin LPL activity were observed in the rat, man, dog, and coypu. In the rabbit and the guinea pig, lower levels of post-heparin serum LPL activity developed. In some guinea pigs no LPL activity was detectable after heparin injection. The effect of heparin added in vitro in increasing concentrations to the LPL assay system showed three contrasting results: a marked increase in LPL activity in the rat, a moderate depression in man, the coypu, the rabbit, and the dog, and no effect in the guinea pig. Peak LPL activity was observed in the rat at an in-vitro heparin concentration of 1.0 U/ml. At 10 U/ml, less stimulation was observed, possibly because of alteration of the apoenzyme or the substrate at this high concentration. The lack of stimulation of LPL activity in all species except the rat may be due to the fact that the circulating LPL is fully activated by heparin and no free apoenzyme is present to be stimulated by heparin addition. Alternatively, these animals may have a deficiency of a specific serum activation factor that is present only in rat serum.

When we added whole rat serum to the assay system in the presence of increasing concentrations of heparin in experiments 2 through 6, the stimulation of LPL activity by heparin in the presence of rat serum throughout the entire range of in-vitro heparin concentrations in the assay system indicates that rat serum contains a unique factor for activating serum LPL. Rat serum increased guinea pig LPL activity by 2,700% at an in-vitro heparin concentration of 1.0 U/ml. Human serum added to the guinea pig LPL assay system was just as effective as rat serum in the absence of in-vitro heparin, but in the presence of 1.0 U/ml heparin, the stimulation was only 500%. Thus, the manner in which human and rat serum interact with the heparin-LPL system is different. In a separate experiment, it was determined that the percent stimulation of guinea pig post-heparin serum LPL activity is proportional to the rat serum concentration in the assay system (unpublished observations). The results of the experiments with the guinea pig suggest that guinea pig serum is almost totally deficient in a serum factor that can be supplied by small amounts of rat serum. These observations raise the question of how the guinea pig is able to assimilate chylomicrons or very low-density lipoproteins that enter the circulation. One explanation is that the intestinal mucosa or the liver may produce the serum factor necessary for assimilation. If this
factor had a very rapid turnover rate, its concentration in serum at any one time could be very low, and this could account for the inability of guinea pig serum to effectively activate the triglyceride emulsion.

Explanation for our results in the guinea pig may lie in the experimental results of Puppione, who found an absence of detectable serum high-density lipoprotein in the guinea pig (11). In contrast, he found that in the Buffalo rat the high-density lipoprotein distribution as determined on the analytical ultracentrifuge has a fast-moving peak of 6 to 7 F units and widespread distribution throughout the entire flotation range of high-density lipoprotein. On the other hand, in rabbit, man, and dog, he found a narrow distribution with a peak of 2.5 to 3.5 F units. Patterns of high-density lipoprotein determined on Long-Evans rats used in our studies showed a pattern similar to that of the Buffalo rat. However, the pattern of the coypu (another member of the order Rodentia) had a peak of 2.5 to 3.5. These observations indicate that rat serum has a unique high-density lipoprotein pattern, and this fraction may contain the factor responsible for our results. It may in fact be one of the subfractions of high-density lipoprotein. Koga, Horwitz, and Scanu studied the high-density lipoprotein of the rat, which appeared physically homogeneous by the analytical ultracentrifuge (flotation and sedimentation studies, sedimentation equilibrium) but was heterogeneous by the immunological and electrophoretic techniques employed. This suggests the possibility that rat high-density lipoprotein is composed of distinct lipoprotein subgroups of similar density in which the protein moieties differ both chemically and immunologically (12).

Mechanisms of lipoprotein lipase activation are suggested by the data presented. A serum high-density lipoprotein fraction or a subfraction appears to be essential for the function of lipoprotein lipase. The high-density lipoprotein may facilitate the interfacial attachment of the apoenzyme-heparin complex to its triglyceride substrate with heparin serving as an apoenzyme modifier. Alternatively, the high-density lipoprotein may act as a direct allostERIC effector of the lipoprotein lipase or it may function with heparin in bridging the apoenzyme to its substrate. Rat serum contains an active component which may be a high-density lipoprotein fraction that facilitates the lipoprotein lipase activation by heparin because of undefined molecular characteristics.

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