Heparin-Induced Release of Lipase Activity From Perfused Canine Subcutaneous Adipose Tissue[1] (35816)

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Mast cells store histamine, heparin, and a specific protein in specialized storage granules (1). Recently, it has been shown that agents such as Compound 48/80 and *d*-tubocurarine, which cause degranulation of rat mast cells *in vitro*, induce histamine release in canine subcutaneous adipose tissue with a concomitant increase in lipolysis (2). The increase in lipolysis is probably secondary to histamine release, since this amine evokes lipolysis in adipose tissue both *in vivo* (3, 4) and *in vitro* (2, 5). Due to their histamine content, mast cells may participate in the regulation of fat metabolism in adipose tissue. It has been implied that mast cells play a role in lipid metabolism because of their content of heparin (6).

Heparin has been shown to cause release of an enzyme, similar to clearing factor lipase (CFL)[4] from the perfused rat epididymal fat pad (7) and to decrease the amount of CFL extractable from this tissue (8). There is evidence suggesting that CFL is of some importance in the regulation of the deposition of triglycerides in tissues (9). Therefore, heparin, histamine, and Compound 48/80 were studied with regard to their ability to influence the release of lipase activity into the perfusate from canine subcutaneous adipose tissue.

Methods. Female mongrel dogs anesthetized with sodium pentobarbital (30 mg/kg, iv) were used. Supplemental injections of sodium pentobarbital were given when necessary during experiments. The subcutaneous adipose tissue in the inguinal region was isolated from the surrounding skin and muscle (10) and all vessels except the main artery and vein were ligated. The adipose tissue was denervated. The tissue was perfused from a reservoir which delivered defibrinated blood at a constant rate (11). Venous outflow was measured with a drop chamber and collected at timed intervals into tubes placed in crushed ice. Systemic blood pressure was measured from the femoral artery with a Statham transducer.

Aliquots of the venous blood were centrifuged and plasma samples were taken for determination of FFA (12) and glycerol (13). Arterial blood samples were taken from the reservoir at the beginning and end of the experiments and plasma FFA and glycerol were also measured. The mean value was taken as the arterial concentration of these substances. Another aliquot of venous blood plasma was stored overnight at 4° for the assay of lipolytic activity according to Boberg and Carlson (14) with slight modifications: To 3.85 ml of a 0.15 *M* phosphate buffer (pH 7.4), 0.15 ml of Intralipid[5] was

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[4] The term "clearing factor lipase" is used in preference to "lipoprotein lipase" in this communication. The assay method used in this study is not specific for lipoprotein lipase. Although the assay predominately measures lipoprotein lipase activity (glyceryl triester hydrolase), monoglyceridase activity cannot be excluded.

[5] Intralipid, an emulsion containing 10 g of soybean oil, 1.2 g of purified egg phosphatides and 2.5 g of glycerol, was kindly supplied by AB Vitrum, Stockholm. Unlike Ediol, Intralipid does not contain monoglyceride. Therefore, Intralipid is a suitable sub-

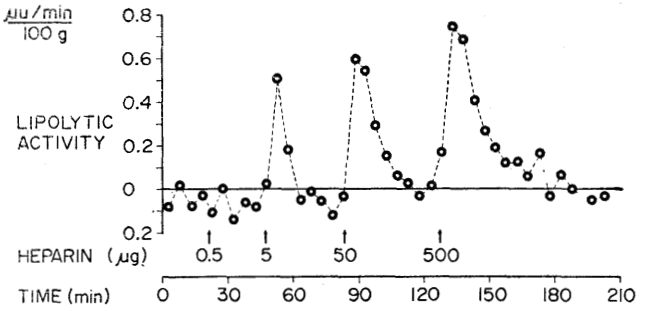

FIG. 1. The effect of intra-arterial injections of 0.5, 5, 50, and 500 μg of heparin on the release of venous plasma lipolytic activity (VPLA) from canine subcutaneous adipose tissue at a constant rate (2.1 ml/min/100 g) with defibrinated blood. The adipose tissue weight was 47 g.

added. One milliliter of plasma was then added to the substrate mixture, mixed, and incubated for 60 min at 37°. One milliliter of the assay mixture was pipetted immediately and at the end of 60 min of incubation for FFA analysis in duplicates. Under the above conditions, 1 μU of lipolytic activity equals the production of 1 μmole of FFA/60 min.

Results. Effect of heparin. Single intra-arterial injections of 0.5 to 500 μg of heparin were given to five dogs. The lowest dose (0.5 μg) had no effect on the venous plasma lipolytic activity (VPLA). Heparin in amounts of 5 μg or greater showed progressive increases in lipolytic activity (Fig. 1).

Figure 2 shows the VPLA release following five consecutive injections of 50 μg of heparin. The first three, administered at intervals of 50 min, were equally effective. A fourth dose given 35 min after the preceding one was less effective. Injections of 50 and 500 μg given at 15 min, were relatively ineffective. The release of FFA (Fig. 2) or glycerol (not shown in Fig. 2) was not enhanced by the injection of heparin.

Continuous infusion of heparin at a constant rate (500 μg/ml of plasma, ia) for 140 min produced an initial increase in VPLA, but the effect was not maintained beyond 40 min (Fig. 3). However, the activity remained somewhat above the basal levels throughout the remaining period of perfusion.

Effects of histamine and Compound 48/80. Histamine, in doses of 2, 25, and 250 μg, was administered intra-arterially in two experiments. No increase in VPLA was observed. Similarly, Compound 48/80 in doses of 50 and 100 μg given to six dogs, failed to increase the VPLA. However, both histamine and Compound 48/80 increased the release of FFA and glycerol as reported previously (3).

Discussion. The finding that heparin increased the release of lipase activity into the venous blood plasma is in agreement with the observations of Ho *et al.* (7). They reported that the lipase activity released from the isolated perfused rat epididymal fat pad by heparin is dissociated from the catecholamine-sensitive enzyme which hydrolyzes intracellular triglycerides resulting an increase in FFA and glycerol mobilization. The results of the present study that heparin had no effect on the rate of glycerol release from the adipose tissue and that sympathetic nerve stimulation did not affect the release of lipase activity (unpublished result) are compatible with the findings of Ho *et al.* (7).

The diminished VPLA release observed after repeated single injections and continued infusion of heparin seems to suggest enzyme depletion from its storage site as proposed by Atkin and Meng (15) and Ho *et al.* (7). Preliminary studies on lipase depletion and repletion in the isolated perfused rat heart have been reported (16), but the mechanisms are not known.

strate for measuring lipolytic activity of plasma after heparin administration. It may also be possible that postheparin plasma can hydrolyze phospholipid which may render the finding of FFA somewhat higher than that from hydrolysis of triglycerides.

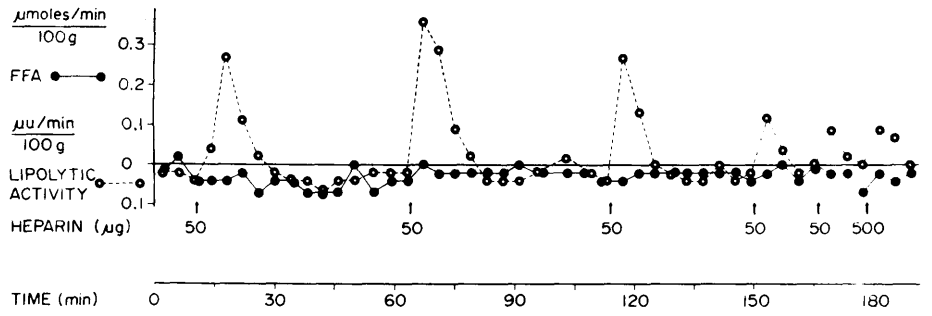

FIG. 2. The effect of intra-arterial injections of 50 and 500 μg of heparin on the release of VPLA and FFA from canine subcutaneous adipose tissue (46 g) perfused at a constant rate (3.2 ml/min/100 g) with defibrinated blood.

It is interesting that Compound 48/80 did not increase the release of lipase activity from canine subcutaneous tissue. However, this agent was found effective in the release of lipase from the isolated rat heart (17). It has been reported (18) that mast cells, treated with Compound 48/80, release their storage granules into the extracellular fluid. Since rat mast cell granules contain about three times more heparin than histamine on a weight basis (A. Bergendorff, personal communication) and 50 μg of Compound 48/80 was found to release about 20 μg of histamine from the adipose tissue (3), then the amount of heparin extruded from mast cells under the same circumstances should be sufficient to cause VPLA release. There may be several explanations for the failure of Compound 48/80 to cause VPLA release. The heparin–protein complex, forming the matrix of the mast cell granules, might be insoluble in physiological media so that no free heparin is formed. It is also conceivable that heparin is released at a site where it does not come in contact with releasable lipase. Thus, the results of the present study seem to suggest that mast cells in canine adipose tissue may have a function in FFA mobilization due to their histamine content, but failed to provide evidence for a role of its heparin in the release of lipolytic activity.

Summary. The effects of heparin, histamine, and Compound 48/80 on the release of free fatty acids (FFA), glycerol, and venous plasma lipolytic activity (VPLA) from the isolated perfused canine subcutaneous adipose tissue *in situ* was studied. Intra-arterial administration of heparin with dosages be-

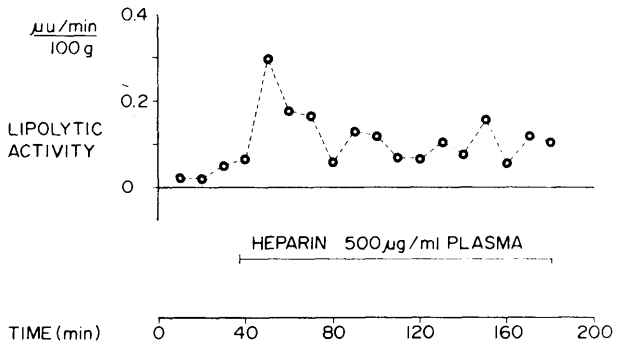

FIG. 3. The effect of the infusion of 500 μg of heparin/min on the release of VPLA from canine subcutaneous adipose tissue (82 g) perfused at a constant rate (1 ml/min/100 g) with defibrinated blood. The large variation in the release of VPLA from the perfused tissues of different animals has been observed in this study. The small effect of heparin administration on the release of VPLA in this experiment as compared to that shown in Fig. 1 is, in part, due to this variation. In addition, the perfusion flow rate was fairly low in this experiment.

tween 5 to 500 μg produced an increase in VPLA release which was dose dependent. The VPLA release was decreased after repeated injection of heparin. Heparin administration did not alter the release of FFA and glycerol. Histamine and Compound 48/80 increased FFA and glycerol release, but produced no effect on VPLA release. The results do not support the contention concerning a possible role of mast cell heparin in the regulation of lipid metabolism in canine subcutaneous adipose tissue but confirm the previous findings that mast cell histamine may exert some effect in the mobilization of FFA.

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