STUDIES OF ENDOTOXIN-INDUCED DECREASE IN LIPOPROTEIN LIPASE ACTIVITY*

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Similar physiological and biochemical responses are induced by a variety of invasive stimuli including bacterial, viral, protozoan and metazoan infection, and some tumors. These include fever, leukocytosis, and elevated concentrations of specific serum proteins such as fibrinogen, haptoglobin (1, 2), and serum amyloid A protein (SAA)* (3, 4). Recently, we noted that an additional biochemical response, a hypertriglyceridemia, characteristically occurs in rabbits infected with the protozoan parasite, Trypanosoma brucei (5). This hypertriglyceridemia, representing an accumulation of very low density lipoprotein in plasma, was found to result from decreased removal due to a deficiency of lipoprotein lipase, the key enzyme of triglyceride metabolism (6) in the peripheral tissue. A similar biochemical derangement was observed in rabbits carrying the V-2 carcinoma (C. A. Rouzer and A. Cerami, unpublished observations). Hyperlipidemia has also been associated with several bacterial (7, 8) and viral infections (9, 10) and with endotoxemia (11-13). The generalized occurrence of this phenomenon stimulated us to investigate the biochemical mechanism(s) responsible for the lipoprotein lipase deficiency.

The hypertriglyceridemia associated with the administration of bacterial endotoxin was chosen as an experimental model because it is simpler and more quantifiable than experimental infection. Furthermore, endotoxin is a well-characterized material. The endotoxin model has the significant additional advantage of having two genetically similar strains of mice, one of which is sensitive to and one of which is resistant to this material (14). Using these endotoxin-sensitive and -resistant mice, previous workers (15, 16) have demonstrated that several biological actions of endotoxin are mediated by exudate cells. These include granulocytosis, changes in plasma iron and fibrinogen concentration, and production of SAA. It appears that endotoxin interacts with these exudate cells, causing the release of a humoral mediator factor that is responsible for the effect observed. In the present communication we report that the endotoxin-induced decrease in lipoprotein lipase activity proceeds by a similar mechanism. Exudate cells, in response to stimulation by endotoxin, release a mediator substance that causes a lowering of lipoprotein lipase activity in adipose tissue.

Materials and Methods

Endotoxin. Endotoxin (lipopolysaccharide) from Escherichia coli 0127:B8 isolated by the method of Westphal (17) was purchased from Difco Laboratories, Detroit, Mich.

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1 Abbreviations used in this paper: DME, Dulbecco's modified Eagle's medium; LPL, lipoprotein lipase; SAA, serum amyloid A protein.
Mice. Male C3H/HeN mice (7-10 wk old, 18-25 g) were obtained from Charles River Breeding Laboratories, Wilmington, Mass. Male C3H/HeJ (7-10 wk old, 18-25 g) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Mice were fed ad libitum on Rodent Laboratory Chow (Ralston Purina Co., St. Louis, Mo.) until they were used. The chow diet was removed 24 h before the experiment and replaced with a solution of 25% sucrose in water. Once injected, the animals were only allowed access to water. 3-10 C3H/HeN or C3H/HeJ mice were used for each experimental group.

Each mouse was injected intraperitoneally with one of the following: (a) 0.04-100 μg of endotoxin; (b) 0.5 ml of serum obtained from C3H/HeN mice treated with endotoxin or saline; or (c) 1 ml of medium from cultures of peritoneal exudate cells of mice incubated in the presence or absence of endotoxin. Animals were killed at the indicated times by decapitation. The serum triglyceride concentration and lipoprotein lipase (LPL) activity in the heart and the epididymal fat pads were determined.

Assay for Serum Triglyceride Concentration and Tissue LPL. The triglyceride concentration was measured with an enzymatic assay (triglyceride test set 961; Hycel, Inc., Houston, Tex.). LPL activity was assayed by the previously described methods with some modifications (18, 19). Epididymal fat pads and heart were excised immediately after the decapitation of each mouse. The tissues were rinsed in sterile Dulbecco's modified Eagle's medium (DME) (Grand Island Biological Co., Grand Island, N.Y.) containing 2% bovine serum albumin (fraction V; Reheis Chemical Co., Phoenix, Ariz.) and blotted on sterile filter paper. The tissues were minced with scissors, put into preweighed sterile polystyrene culture tubes (17 × 100 mm; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) containing 1 ml of DME medium supplemented with 2% bovine serum albumin, and 2 U of heparin (Lipo-Hepin; Riker Laboratories Inc., Northridge, Calif.). Tubes with the tissues were sealed under 5% CO₂ in air, and incubated at room temperature with continuous gentle shaking. Tissue weight was determined by the difference of the weights of the tube before and after the addition of the tissue. Approximately 100-300 mg of tissue was used for each assay. After 2.5 h incubation, an aliquot of heparin-DME medium was removed and the activity of LPL released from the tissue was determined. The enzyme assay was carried out by the method of Nilsson-Ehle and Schotz (20) with minor modifications. The samples were incubated at 37°C for 90 min with a triglyceride emulsion. The release of free fatty acid was linear up to 120 min of incubation. The hydrolytic activity was inhibited >80% if 1 M NaCl was added to the assay. Each sample was assayed in duplicate. One milliunit of the enzyme activity was defined as one nanomole of free fatty acid released per minute. The enzyme activity released per gram of wet tissue was compared between experimental groups and control groups for each study because there was considerable variation in LPL activity from day to day. To compare the data between experiments, the data are expressed as the percent of the average activity of the control group. The range observed in C3H/HeN mice was from 32 to 59 mU/g for adipose tissue and 320 to 836 mU/g for the heart. Values of 31 to 172 mU/g for adipose tissue and 191 to 504 mU/g for the heart were observed in C3H/HeJ mice.

Collection of Serum from Endotoxin-treated Mice. Blood was obtained under sterile conditions from the axillary pit of C3H/HeN mice 2 h after intraperitoneal injection of endotoxin (either 2 or 100 μg/mouse) in 0.2 ml of saline or saline alone. Serum was prepared within 1 h after bleeding and either used immediately or kept at −80°C until use.

Preparation of Endotoxin-treated Peritoneal Exudate Cells. Peritoneal exudate cells were obtained by peritoneal lavage with pyrogen-free saline (Abbott Diagnostics, Diagnostic Products, North Chicago, Ill.) from C3H/HeN mice (25-35 g). These mice were injected intraperitoneally 6 d before lavage with 3 ml of sterile Brewer's thioglycollate medium (Difco Laboratories). The peritoneal exudate cells obtained with this procedure consist of ~60% macrophages, 20% small lymphocytes, 15% large lymphocytes, and 5% eosinophils (21).

The exudate cells (2 × 10⁶ cells/well) were incubated in serum-free RPMI 1640 medium (Grand Island Biological Co.) in culture plates containing 4.5-cm² wells (Flow Laboratories, Inc., Linbro Chemical Co., Hamden, Conn.) at 37°C in 5% CO₂. After 3 h, the cultures were washed three times with the medium to remove nonadherent cells. The cells that adhered to the dish were mainly macrophages. The cells were incubated further in serum-free RPMI 1640 medium in the presence or absence of endotoxin (10 μg/ml). The culture medium was removed
after 26 h incubation and centrifuged at 1,000 g for 5 min at 4°C. The supernate was used immediately or kept at -80°C until use. No difference in activity was noted after storage for 1 mo under these conditions.

Results and Discussion

Effect of Endotoxin on Serum Triglyceride Concentration and LPL in Mice. Fig. 1 shows the activity of LPL from adipose tissue (epididymal fat pads) and serum triglyceride concentration of endotoxin sensitive mice, C3H/HeN, which had been injected with either saline or 100 µg of endotoxin 16 h before killing. This amount of endotoxin corresponds in this strain of mice to a dose in which half the animals die within 3 d after the injection. The LPL activity of adipose tissue in the endotoxin-treated animals was markedly depressed (4.3% of the control value), whereas the triglyceride concentration in the serum of the endotoxin-treated animal was elevated 2.6 times that of control animals. The hypertriglyceridemia and the reduction of LPL in adipose tissue had not yet occurred 12 h after the injection of endotoxin. At 24 h, the depression of LPL activity was still marked (2.0% of the control value), whereas the triglyceride concentrations had returned to normal in four of the six mice injected with endotoxin (data not shown). This normalization of serum triglyceride levels at 24 h could reflect decreased very low density lipoprotein production and/or a compensatory increase in LPL activity by other tissues. In all subsequent experiments, LPL activity was determined 16 h after endotoxin administration.

![Fig. 1. Effect of endotoxin on serum triglyceride (TG) and adipose tissue LPL activity in endotoxin-sensitive mice. 100 µg of endotoxin dissolved in 0.2 ml of saline was injected into C3H/HeN mice. Serum and tissue were obtained 16 h after the injection. Plasma triglyceride and tissue LPL were assayed as described in Materials and Methods. Control mice were injected with pyrogen-free saline. Data are expressed as the mean (± SEM) of six mice for each group.](image)
Fig. 2a records the effect of increasing amounts of endotoxin on LPL activity in adipose tissue of C3H/HeN and C3H/HeJ mice. It is apparent that the endotoxin-resistant (C3H/HeJ) mice did not have as low LPL activity after endotoxin admin-
istration as did the sensitive mice (C3H/HeN). The ED_{50}, a dose that causes a 50% decrease in LPL activity, was 1 µg and 0.04 µg in C3H/HeJ and C3H/HeN mice, respectively.

LPL activity in the heart was not as markedly affected as enzymatic activity in adipose tissue, even in the endotoxin-sensitive C3H/HeN mice (Fig. 2b). The administration of 2 µg of endotoxin (a dose that reduced enzyme activity in adipose tissue by 88%) had no effect on the enzyme activity of the heart. Larger amounts of endotoxin did have noticeable effects on heart LPL activity, however.

_Effect of Serum from Endotoxin-treated C3H/HeN Mice._ To determine whether the suppressive effect of endotoxin on LPL activity of adipose tissue was mediated by a humoral factor, we obtained serum from endotoxin-sensitive C3H/HeN mice that had been injected with 100 µg of endotoxin 2 h before bleeding. This serum was then injected into another group of C3H/HeN mice. The control group was injected with serum obtained from C3H/HeN mice treated with pyrogen-free saline. LPL activity in epididymal fat pads was measured 16 h later. As shown in Fig. 3a, the serum from endotoxin-treated mice markedly suppressed LPL activity in these animals compared with the activity observed in the control group of animals. Because >90% of endotoxin is cleared from the circulation in 15 min (22, 23), it is unlikely that an observed effect on LPL activity is due to a direct effect of any remaining endotoxin present in the serum 2 h after injection.

**Fig. 3a.** Effect of serum from endotoxin-sensitive mice treated with endotoxin on adipose tissue LPL activity in endotoxin-sensitive mice. 100 µg of endotoxin was injected into a group of C3H/HeN mice. Serum was obtained from these animals 2 h after endotoxin injection and administered intraperitoneally to another group of C3H/HeN mice. For a control group, the serum from the same strain of mice not treated with endotoxin was given. LPL activity was assayed 16 h after serum injection. The data are expressed as the mean (± SEM) of six animals for each group.

**Fig. 3b.** Effect of serum from endotoxin-sensitive mice treated with endotoxin on adipose tissue LPL activity in endotoxin-resistant mice. Serum obtained from C3H/HeN mice treated as previously with 2 µg of endotoxin was injected into C3H/HeJ mice. For a control group, the serum from C3H/HeN mice not treated with endotoxin was given. LPL activity was assayed 16 h after serum injection. The data are expressed as the mean (± SEM) of three animals for each group.
To further exclude direct endotoxin effects, serum obtained from the sensitive C3H/HeN strain, which had been injected 2 h earlier with a smaller amount (2 μg) of endotoxin, was injected into endotoxin-resistant C3H/HeJ mice. The LPL activity of adipose tissue was measured 16 h after the injection to minimize the possibility of direct endotoxin effect and revealed a 55% decrease of LPL activity (Fig. 3b). Because resistant animals do not respond to this small amount of endotoxin, this observation suggests that a humoral mediator is involved to which the resistant mice are capable of responding.

Production of an LPL-suppressing Mediator by Exudate Cells. Experiments were undertaken to determine whether exudate cells could be stimulated to produce the mediator by which endotoxin suppresses the LPL activity of adipose tissue. Exudate cells were obtained from endotoxin-sensitive (C3H/HeN) mice by peritoneal lavage. These cells were incubated in vitro in the presence (10 μg/ml) or absence of endotoxin. 1 ml of the media from these cell cultures was injected into the endotoxin-resistant strain C3H/HeJ mice. As displayed in Fig. 4, the average LPL activity in adipose tissue of animals injected with medium from the exudate cells incubated with endotoxin was 32% of that of mice that received either medium from cell cultures without added endotoxin or medium containing endotoxin without cells. The difference in enzyme activity between animals treated with medium from endotoxin-treated cell cultures and those animals treated with saline alone was much greater than the other controls, suggesting that a small amount of mediator was released by exudate cells in the absence of endotoxin and that the small amount of endotoxin in the medium without cells was enough to partially lower LPL activity. In contrast to the marked depression
of LPL activity in adipose tissue, the LPL activity in the heart was not significantly affected by medium from endotoxin-treated exudate cell cultures (data not shown).

From the experimental data presented, it is clear that endotoxin administration markedly suppresses adipose tissue LPL in genetic strains of mice that are sensitive to endotoxin shock and death. This action is mediated by a humoral factor or factors that can suppress adipose tissue LPL in mice not sensitive to endotoxin shock as well as in mice that are sensitive. Peritoneal exudate cells sensitive to endotoxin appear to be involved in the production of this humoral mediator. The observed time lag (14 h) between mediator release and a decreased LPL activity presumably reflects the turnover time of this enzyme.

At present, the chemical nature of the mediator is not known. Whether it is identical to or distinct from previously described endotoxin-induced exudate cell mediators (15, 16) remains to be determined. It is also unclear whether decreased LPL activity in response to endotoxin and other insults to the organism serves a specific protective function. It may simply reflect the production by sensitive exudate cells of a humoral mediator whose major biological role lies elsewhere. In either case, however, greater understanding of the mechanism by which sensitive-exudate cells decrease LPL would seem to have much relevance for other disease states associated with LPL deficiency such as familial hypertriglyceridemia (24), diabetes mellitus, and obesity (25). Further work is necessary to clarify these important questions.

Summary

A variety of invasive stimuli have been shown to induce hyperlipidemia due to impaired removal of triglyceride from the circulation. The mechanism by which endotoxin induces a deficiency in the activity of the key enzyme of triglyceride metabolism, lipoprotein lipase (LPL), has been studied. In C3H/HeN (endotoxin-sensitive) mice, LPL activity in adipose tissue was markedly suppressed 16 h after endotoxin administration. In contrast, the endotoxin-resistant C3H/HeJ mice were less sensitive to the suppressive effect of endotoxin on LPL activity. After endotoxin administration, a transferrable factor had been detected in the blood of C3H/HeN mice 2 h after the injection of endotoxin that causes a suppression of adipose tissue LPL activity in C3H/HeJ mice as well as in C3H/HeN mice. Conditioned medium from the cultures of peritoneal exudate cells of C3H/HeN mice incubated in endotoxin also suppresses adipose tissue LPL in C3H/HeJ mice. These studies demonstrate that exudate cells produce a humoral factor in response to endotoxin, which suppresses adipose tissue LPL.

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