Streptozotocin-Induced Diabetes Enhances Cardiac Heparin-Releasable Lipoprotein Lipase Activity in Spontaneously Hypertensive Rats

Greg Shepherd, Margaret C. Cam, Nandakumar Sambandam, Mohammed A. Abrahani, Brian Rodrigues

Abstract—Vascular endothelial-bound lipoprotein lipase (LPL), also known as heparin-releasable LPL, catalyzes the breakdown of the triglyceride component of lipoproteins and is rate-limiting for free fatty acid transport to tissues. We previously demonstrated that heparin-releasable LPL activity increases in diabetic Wistar rat hearts, whereas with the development of hypertension in spontaneously hypertensive rats (SHR), there is a concomitant and progressive reduction in LPL activity. The objective of the present study was to examine the regulation of cardiac LPL activity in SHR-diabetic rats. Heparin perfusion of the isolated Langendorff heart induced the release of LPL activity. SHR hearts demonstrated a reduction in peak heparin-releasable LPL activity, relative to Wistar controls. However, induction of streptozotocin-induced diabetes in SHR, as in Wistar rats, increased peak heparin-releasable LPL activity in perfused hearts. The elevated heparin-releasable LPL peak could not be accounted for by enhanced LPL synthesis in that both cellular and surface-bound LPL activities in myocytes from SHR-diabetic rats were low relative to control. Chronic (12-day) insulin treatment of SHR-diabetic rats reduced the augmented heparin-releasable LPL activity and increased cell-associated LPL activity. Moreover, acute (90-minute) treatment of SHR-diabetic rats with rapid-acting insulin also reduced the heparin-releasable LPL activity to normal, although it had no effect on the low cellular LPL activity. These results demonstrate that the diabetes-induced augmentation of cardiac LPL counteracts the reduction in enzyme activity associated with hypertension. This may serve to increase the delivery of free fatty acid to the heart, and the resultant metabolic changes may lead to the severe cardiomyopathy observed in the hypertensive-diabetic rat heart. (Hypertension. 1998;31:878-884.)

Key Words: lipoprotein lipase ■ rats, inbred SHR ■ streptozotocin ■ diabetes ■ cardiomyopathy ■ heparin

Increasing evidence suggests that an altered substrate supply and utilization by cardiac myocytes could be the causal injury in the pathogenesis of diabetic cardiomyopathy.1-4 Importantly, in diabetes, glucose utilization is insignificant, and energy production is shifted almost exclusively toward β-oxidation of FFA.5 FFAs are supplied to cardiac cells from endogenous cardiac TG stores or from exogenous sources in the blood (as free acid bound to albumin or as TG in lipoproteins). Vascular endothelium-bound LPL catalyzes the breakdown of the TG component of VLDL and chylomicrons and regulates tissue FFA supply; it is also called “functional” LPL.6 In the adult heart, LPL is synthesized and processed in myocytes and subsequently translocated onto HSPG binding sites on the luminal surface of endothelial cells.7 At this location, it actively metabolizes lipoprotein TG to FFA and 2-monoacylglycerol,8 which are transported into the heart for numerous metabolic and structural tasks. Recently, it was demonstrated that LPL activity, rather than FFA transport via the endothelial cell or sarcotemma, is the rate-limiting step in the cellular uptake of TG-derived FFA.9

The approximate contribution of endogenous or exogenous sources of FFA toward β-oxidation in the diabetic heart is unknown. In an insulin-deficient state, hydrolysis of augmented myocardial TG stores could contribute to tissue FFA.10-12 In addition, adipose tissue lipolysis is enhanced, resulting in elevated circulating FFA.13 However, changes in cardiac LPL activity, which controls exogenous FFA uptake, are inconsistent.14-16 Recently, we demonstrated an elevated heparin-releasable LPL activity in diabetic rat hearts,17 which we hypothesized could increase FFA supply and utilization by cardiac cells.

Hypertension occurs more frequently in type 118 and type 219 diabetic patients than in the nondiabetic population of the same age and gender. An important feature of the hypertensive-diabetic condition is its synergistic effects on the development of heart failure.20 Induction of STZ-induced diabetes in the SHR also produces a more extensive cardiomyopathy and an increased mortality rate.21 We previously reported that coinciding with the onset of hypertension in the SHR is a progressive reduction in heparin-releasable endothelial LPL.22

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technique until the perfusate was clear of blood.26 This period is

necessary to remove proteases released by tissue damage during the
dissection that would normally reduce LPL activity subsequently
measured in the hydrolysis assay. The perfusion fluid was continuously
gassed with 95% O2/5% CO2 in a double-walled water-heated
chamber maintained at 37°C with a temperature-controlled circulat-
ing water bath. A peristaltic pump controlled the rate of coronary flow
(7 to 8 mL/min). To measure the release of LPL activity into the
medium, the perfusion solution was changed to Joklik containing 1% BSA
(Fraction V; Boehringer-Mannheim Biochemica), 1 mmol/L CaCl2, and heparin (5 U/mL).27 This concentration of heparin was previously
shown to maximally release cardiac LPL from its binding site.23 The coronary effluent was collected in timed fractions and
frozen until assayed for LPL activity.

Preparation of Cardiac Myocytes
Perfusion of the heart with heparin predominantly releases extracel-
ular, endothelium-bound LPL; however, activity is still measurable
within the heart. This heparin-nonreleasable LPL activity is located
predominantly within the myocytes. To measure this fraction, calci-
um-tolerant myocytes were prepared from hearts (ventricles) accord-
ing to a previously described procedure.26 Briefly, hearts were re-
moved from anesthetized rats and digested through perfusion of
collagenase (225 U/mL) retrogradely through the heart. Myocytes
were made calcium tolerant by successive exposure to increasing
concentrations of calcium. Our method of isolation yields a highly
enriched population of calcium-tolerant myocardial cells that are rod
shaped in the presence of 1 mmol/L CaCl226 with clear cross-striations.
Intolerant cells are intact but hypercontract into vesiculated spheres.
Yield of myocytes (cell number) was determined microscopically
using an improved Neubauer hemocytometer. Myocyte viability
(generally between 75% and 85%) was assessed through trypan blue
exclusion.

Cardiac myocytes from control and diabetic rats were suspended in
Joklik minimum essential medium to a cell density of 0.4×106
cells/mL and incubated at 37°C under an atmosphere of 95% O2/5%
CO2. To release surface-bound LPL activity, heparin (5 U/mL) was
added to the myocyte suspension. Aliquots of cell suspension (1 mL)
were removed at specified intervals, and the medium was separated
from cells by centrifugation (3000g for 10 seconds). The supernatant
was decanted and stored at −70°C until it was assayed for LPL
activity. The corresponding cell pellets were also stored frozen for
measurement of intracellular LPL activity.

LPL Assay
LPL catalytic activity in perfusates and incubation medium of
cardiac myocytes was determined by measuring the in vitro hydrolysis
of a sonicated [3H]triolein substrate emulsion. The standard assay
conditions were 0.6 mmol/L glycerol tri-[9,10-3H]oleate (1 mCi/
mmol; 1 Ci = 37 GBq), 25 mmol/L piperazine-N,N’-bis(2-ethanesul-
fonylic acid) (pH 7.5), 0.05% (wt/vol) albumin, 50 mmol/L MgCl2, 2%
(vol/vol) heat-inactivated chicken serum (containing the LPL activa-
tor apolipoprotein CII), and 100 µL of either medium or heart
perfusate in a total volume of 400 µL. The release of [3H]oleate was
measured after an incubation of 30 minutes at 30°C. Hydrolyzed
[3H]oleate in the medium was determined by the addition of 3 mL of
a fatty acid extraction solution (methanol 38.5%, chloroform 34.2%,
heptane 27.3%) and 100 µL of 0.1 mol/L NaOH.26 After vortex
mixing and centrifugation, the radioactive sodium ([3H]oleate in a
sample (0.5 mL) of the upper phase was determined by liquid
scintillation counting. All LPL assays were performed in duplicate
under similar conditions in which the reaction rate was linear with respect to
time and the volume of medium that was assayed.28 For the LPL assay,
interassay and intra-assay coefficients of variation (%CV) did not
exceed 10%. Results are routinely expressed as nanomoles of oleate
released per hour per milliliter (coronary perfusate) or 106 cells
(myocyte medium or cells).

Heparin-nonreleasable cellular LPL activity was measured through sonication of (twice at 30 seconds) the cell pellets after resuspending
them in 0.2 mL of 50 mmol/L NH4Cl buffer (pH 8.0) containing
0.125% (vol/vol) Triton X-100. After sonication, the volume was
adjusted to 1 mL with use of a sucrose buffer (0.25 mol/L sucrose,
Cardiac LPL in Hypertensive-Diabetic Rats

Effect of Chronic (2-Week) Insulin Treatment on Characteristics of SHR Made Diabetic With 55 mg/kg STZ

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>STZ Injected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td>Fluid intake, mL/d</td>
<td>36±1</td>
<td>126±4*</td>
</tr>
<tr>
<td>Plasma insulin, ng/mL</td>
<td>4.0±0.3</td>
<td>0.6±0.1*</td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td>8.0±0.3</td>
<td>23.4±0.8*</td>
</tr>
<tr>
<td>Plasma TG, mmol/L</td>
<td>0.7±0.1</td>
<td>1.5±0.2*</td>
</tr>
<tr>
<td>Plasma cholesterol, mmol/L</td>
<td>1.37±0.04</td>
<td>1.55±0.09</td>
</tr>
<tr>
<td>Ventricle-to-body weight ratio, mg/g</td>
<td>3.96±0.08</td>
<td>3.70±0.16</td>
</tr>
<tr>
<td>Cell viability, % rod-shaped cells</td>
<td>78.6±1.3</td>
<td>81.6±1.4</td>
</tr>
<tr>
<td>Cell yield, ×10^6 rod-shaped cells/heart</td>
<td>5.9±0.8</td>
<td>5.8±1.0</td>
</tr>
</tbody>
</table>

Results are mean±SEM of 6 to 8 animals in each group. Values were obtained before death.

Results are significantly different from all other groups (P<.05).

Blood pressure in both CON and DIA at 9 to 10 weeks of age increased progressively over 2 weeks relative to WisC and WisD groups. Moreover, diabetes per se had no effect on blood pressure in either rat strain. Other characteristics normally associated with diabetes, such as polydipsia (Table) and hyperphagia (Fig 1), were observed in DIA rats. Despite a greater food intake, DIA rats demonstrated a significantly reduced body weight gain over 2 weeks (Fig 1). Plasma insulin levels were reduced at 2 weeks after diabetes induction, accompanied by marked hyperglycemia (Table). TG levels were elevated in DIA rats, but there was no change in plasma cholesterol levels (Table).

Coronary Endothelial LPL

Retrograde perfusion of whole hearts from CON and 2-week DIA rats with heparin released LPL activity into the coronary perfusate (Fig 2). The heparin-induced LPL discharge in CON rats was rapid, and peak activity, suggested to represent LPL that is located at or near the endothelial cell surface, was observed within 0.5 to 1 minute. Relative to WisC, CON rat hearts demonstrated a decline in peak heparin-releasable LPL activity (Fig 2, right). As previously reported, induction of

Materials

Joklik minimum essential medium was obtained from Gibco Canada. [3H]Triolein was purchased from Amersham Canada. Heparin sodium injection (Hepalean; 1000 USP U/mL) was obtained from Organon Teknika. Collagenase (CLS 2, 325 U/mg) was purchased from Worthington Biochemical Corp. All other chemicals were obtained from Sigma Chemical.

Statistical Analysis

All data are reported as mean±SEM unless otherwise stated. One-way analysis of variance followed by the Newman-Keul’s test or the unpaired Student’s t test was used to determine differences between group mean values. Changes in heparin-releasable and cellular LPL activity in response to heparin over time were analyzed with multivariate analysis of variance followed by the Newman-Keul’s test using the Number Cruncher Statistical System. The level of statistical significance was set at P<.05.

Results

General Characteristics

Induction of diabetes in SHR resulted in glycosuria (>2%) throughout the study period. We previously demonstrated that
STZ diabetes caused an increase in plasma glucose levels (WisC, 6.4±0.3; WisD, 20.2±0.65). Moreover, peak LPL activity in WisD rats was almost twice as much as that in WisC (Fig 2, left). Similar to WisD rats, 2-week DIA rat hearts had a higher peak heparin-releasable LPL activity relative to CON (Fig 2, right).

Myocyte LPL

To determine whether the increase in cardiac heparin-releasable LPL in DIA is a consequence of augmented production, myocytes were isolated at 2 weeks after diabetes induction, and LPL activity in cell sonicates was measured. There was no difference in myocyte viability (percent of live cells) or yield (total number of cells×10⁶) between CON and DIA rats at 2 weeks after the induction of diabetes (Table). DIA rats had a reduced cellular LPL activity compared with CON (Fig 3B). To examine whether the diminished LPL activity in D55 rat myocytes was accompanied by a parallel reduction in the secretion of LPL, myocytes were incubated in the presence of heparin to measure both surface-bound and secreted LPL. There was a significant reduction in heparin-induced release of LPL from cardiac myocytes in DIA rats.

Effects of Insulin Treatment

Chronic insulin treatment of DIA rats for 2 weeks resulted in an increase in body weight and a normalization of food and fluid intake and plasma TG and glucose levels (Figs 1 and 4, inset, and Table). In addition, 2-week insulin treatment attenuated the rise in heparin-releasable (Fig 4A) and prevented the reduction in myocyte secreted LPL activities (Fig 4B). Intravenous injection of DIA rats with rapid-acting insulin reduced hyperglycemia within 90 minutes (Fig 5, inset). Acute insulin treatment also reduced peak heparin-releasable LPL activity to control levels (Fig 5A). However, cell-associated LPL activity remained low in acute insulin-treated DIA rats (Fig 5B).

Dose-Response Effects of STZ

Fig 6 shows the peak heparin-releasable LPL activity in SHR at 2 weeks after the administration of varying doses (25 to 55 mg/kg) of STZ. We previously reported that SHR injected with 25 and 35 mg/kg STZ do not show significant glucose intolerance or hyperglycemia in the fed state. In these rats, peak heparin-releasable LPL activity was unchanged compared with control animals. In contrast, SHR administered 45 and 55 mg/kg STZ demonstrated hyperglycemia in the fed and fasted states. In these animals, peak heparin-releasable LPL activity was equally augmented compared with control animals.

Discussion

In the heart, endothelial LPL at the capillary lumen hydrolyzes lipoprotein TG, providing FFA substrate to cardiac muscle.
The important role of LPL in the hydrolysis of circulating TG is confirmed by the loss of this function when endothelial-bound enzyme is removed by heparin perfusion of the heart. This rapid, heparin-releasable LPL fraction is more sensitive to altered physiological (eg, feeding, fasting) and pathological (eg, diabetes, hyperthyroidism) states than is total cellular activity. During hypertension, LPL activity is reduced in skeletal muscle and adipose tissue in human patients and in Dahl salt-sensitive rats. We recently reported that the heparin-releasable LPL fraction in SHR hearts is reduced relative to that of WKY rats. Because nifedipine and CGS-21680, vasodilators with divergent mechanisms of action, normalized enzyme release, we concluded that flow through coronary blood vessels might influence LPL activity. In this regard, exercise training significantly reduces LPL activity in muscle and adipose tissue as a result of increased blood flow. In the present study, a similar reduction in heparin-releasable LPL was observed in SHR hearts relative to Wistar controls.

Varying levels of cardiac LPL protein or activity have been reported in STZ-diabetic rats. Recently, we demonstrated an enhanced heparin-releasable LPL activity in diabetic rat hearts, even when cellular LPL activity was simultaneously reduced. This enhanced activity was matched by an increase in LPL protein as measured with ELISA. In this study, an elevated peak heparin-releasable LPL activity was also observed in SHR-diabetic hearts. Although the endothelial enzyme is largely derived from cardiac myocytes that synthesize and secrete LPL, the elevated heparin-releasable LPL activity in SHR diabetic rat hearts was associated with a reduced cellular pool. Previously, a decline in cellular LPL catalytic activity was suggested to result from post-transcriptional/translational mechanisms leading to an accumulation of inactive LPL protein in diabetic cardiomyocytes.

In perfused guinea pig hearts, LPL can move from parenchymal cells to the endothelial surface within 30 minutes. In mouse hearts, this movement involves "jumping" of LPL between cell surface–associated heparan sulfate and other polyanions. Thus, the enhanced heparin-releasable LPL pool in SHR-diabetic hearts could involve an accelerated vectorial transfer of LPL from myocytes to the capillary lumen. It should be noted that endothelial LPL can also be derived from circulating LPL, which was demonstrated by the uptake of.
an impairment of the Na\textdollar;td d ylinositol linkage,\textsuperscript{46} cleavage of the glycosylphosphatidylinositol anchor by insulin-sensitive phospholipases could release HSPG and, hence, LPL.\textsuperscript{37,48}

The augmented heparin-releasable LPL activity in the DIA heart could have a number of deleterious effects. An increased enzyme activity could lead to an accelerated hydrolysis of lipoprotein TG, providing excess FFA to the heart. In transgenic mouse lines overexpressing human LPL in skeletal and cardiac muscle, elevated FFA uptake induced a severe myopathy, characterized by muscle fiber degeneration, and extensive proliferation of mitochondria and peroxisomes.\textsuperscript{7} Enhanced FFA metabolism in the heart can inhibit glucose oxidation and increase oxygen requirement.\textsuperscript{49–51} In this regard, it was previously demonstrated that diabetes induces a greater enhancement in FFA utilization in the SHR compared with Wistar rats.\textsuperscript{52} Lipolytic products have also been shown to enhance endothelial permeability.\textsuperscript{53,54} Interestingly, DIA demonstrate greater vascular permeability than nondiabetic SHR, or diabetic normotensive rats.\textsuperscript{55} Finally, LPL has been implicated as a receptor ligand for lipoprotein removal,\textsuperscript{56} thus directly enhancing lipoprotein movement through the artery wall,\textsuperscript{57,58} which in turn can alter Ca\textsuperscript{2+} homeostasis in cardiomyocytes.\textsuperscript{39}

In conclusion, these results demonstrate that the diabetes-induced augmentation of heparin-releasable LPL activity counteracts the reduction in enzyme activity associated with hypertension. We believe that an abnormally high cardiac LPL activity could increase FFA supply, which could lead to metabolic and morphological changes associated with the severe cardiomyopathy observed in this model.

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References


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