PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 168, 395–398 (1981)

Decreased Myocardial Extracellular and Muscle Lipoprotein Lipase Activities in Endotoxin-Treated Rats (41293)

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Abstract. These studies were initiated to determine if skeletal muscle lipoprotein lipase (LPL) activities are decreased as is the case in the myocardium following endotoxin and to compare the distribution of LPL activities between intracellular and extracellular compartments of the heart in endotoxemic and normal rats. Rats were sacrificed 7 hr after an intravenous injection of saline (control) or 2 mg *Escherichia coli* endotoxin per 100 g. LPL activities were measured on homogenates of acetone–ether powders of heart ventricles, epididymal fat pads, and selected skeletal muscles including diaphragm, red gastrocnemius, white gastrocnemius, and soleus. The myocardium from endotoxin-treated rats contained 22% of the LPL activity measured in saline-treated rats. Likewise, red gastrocnemius, soleus and diaphragm muscles from endotoxemic rats had significantly less ($P < 0.05$) enzyme activity than was present in normal rats (42, 57, and 41% of control activity, respectively). Hearts from endotoxin- and saline-treated rats were perfused for 1 min with Krebs–Ringer bicarbonate buffer, pH 7.4, containing 1% BSA and 5 U heparin/ml to release extracellular LPL activity. Endotoxin administration resulted in significantly lower enzyme activities in both intra- and extracellular compartments, but the latter was decreased to a greater extent, being 7% of the corresponding control value. These results indicate that depressed LPL activity following endotoxin injection is widespread among tissues and suggest that the reduction may be most pronounced in the extracellular compartment. The findings support earlier studies implicating impaired triacylglycerol removal as a contributing factor in hypertriacylglycerolemia associated with endotoxicosis.

Several studies have indicated that hypertriacylglycerolemia is prevalent during endotoxicosis and gram-negative bacteremia (1, 2). Kaufmann and associates (3, 4) presented evidence suggesting that increased circulating triacylglycerol concentrations may be the consequence of a depressed ability to remove circulating fat, a process dependent upon extrahepatic lipoprotein lipase (5). Subsequently, it was observed that rat myocardial lipoprotein lipase (LPL) activity was markedly decreased within 7 hr following a single intravenous injection of endotoxin (6). Adipose tissue enzyme activity was also decreased but the response was not as pronounced (6).

The present study was initiated to extend earlier findings concerning alterations in tissue lipoprotein lipase activities during endotoxicosis. Because of its preponderance among the tissues containing lipoprotein lipase, skeletal muscle represents a significant source of the enzyme despite its relatively low activity per weight of tissue when compared to the heart (7). Thus, it became desirable to determine if skeletal muscle lipoprotein lipase activity was altered during endotoxicosis. Since the functionally more important site for tissue LPL activity is that portion in the extracellular compartment, it was also of interest to compare enzyme distribution between the intracellular (heparin nonreleasable) and extracellular (heparin releasable) compartments of isolated perfused hearts from control and endotoxin-treated rats.

Materials and Methods. Male Sprague–Dawley rats (300–350 g) were fasted 24 hr prior to the intravenous injection of 0.5 ml saline per 100 g body wt (control) or 2 mg *Escherichia coli* endotoxin per 100 g in saline while under ether anesthesia. After 7 h rats from both groups were reanesthetized and sacrificed by exsanguination and tissues rapidly excised into cold saline. Heart ventricles, selected skeletal muscle regions, and epididymal fat pads were trimmed, weighed, and delipidated with acetone and ether as previously described (6). The subsequent powder was

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weighed and stored at −50°. Skeletal muscle regions were selected to encompass the three muscle fiber types reported to make up rodent skeletal muscle (8, 9). Thus, the red and white regions of the gastrocnemius and soleus were studied because of their preponderance of fast-twitch-oxidative-glycolytic (FOG), fast-twitch-glycolytic (FG), and slow-twitch-oxidative (SO) fibers, respectively. Diaphragm lipoprotein lipase activity was also measured. Portions of delipidated powders were homogenized in glass tissue grinders (10 mg/ml) in 1.0 *M* ethylene glycol and 0.05 *M* Tris−HCl, pH 8.0, and the homogenate assayed for LPL activity as previously described (6).

The distribution of LPL between the intra- and extracellular compartment was measured in isolated perfused hearts in a second series of rats using methods published earlier (10). Hearts from 7 hr post-endotoxin- or saline-treated rats were perfused (60 cm H_2O, 37°) with Krebs−Ringer bicarbonate (KRB) buffer supplemented with 10 m*M* glucose and 1% bovine serum albumin followed by a 1-min perfusion with a similar medium plus 5 U heparin/ml. In preliminary experiments these conditions were found to optimally release LPL activity from perfused hearts. Enzyme activity in the coronary effluent and remaining in the tissue were measured as described above except homogenization was performed in heparin containing KRB medium instead of the ethylene glycol solution to better match the coronary effluent assay milieu. Preliminary experiments showed little or no difference in the expression of enzyme activity using the two homogenizing media.

In both experiments, statistical differences due to endotoxin were determined by *t* tests with $P < 0.05$ accepted as being different.

Results. Seven hours following the injection of endotoxin heart LPL activity was decreased to 22% of the rate in hearts from time-matched saline-treated rats (Fig. 1). A similar decrease in enzyme activity was also observed in skeletal muscle regions containing a higher percentage of either FOG or SO fibers. Thus, red gastrocnemius

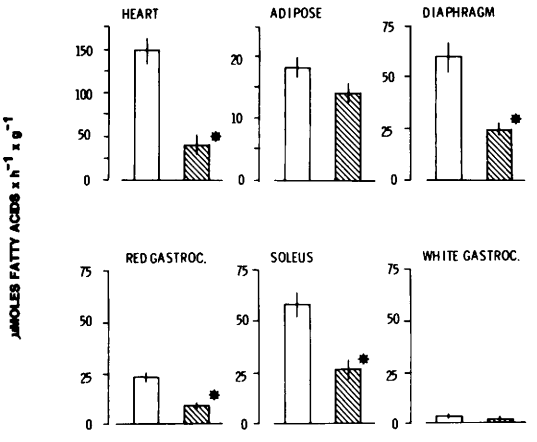

FIG. 1. Tissue lipoprotein lipase activities 7 hr after intravenous administration of saline (open bars) or endotoxin (hatched bars). Data are summarized as $\bar{x} \pm$ SE, $N = 8$. An asterisk indicates significant differences, $P < 0.05$.

and soleus muscle LPL activities in endotoxin-treated rats were 42 and 57% of the activities measured in saline-treated animals, respectively. Also, the diaphragm form endotoxin-treated rats possessed 41% of the enzyme activity present in control animals. Enzyme activity in the white portion of the gastrocnemius muscle was barely detectable. No demonstrable effect of endotoxin on its lipoprotein lipase activity was evident. Adipose tissue enzyme activity was also not significantly reduced by endotoxin administration.

The decreased myocardial LPL activity resulted in nearly complete depletion of activity in its extracellular compartment (Fig. 2). Heart extracellular LPL activity after endotoxin administration was 7% of the corresponding activity measured in saline-treated animals. In addition, heparin non-releasable or intracellular LPL activity was also decreased in the heart of the endotoxemic rat. However, the decrease in the intracellular compartment was not as great as that which took place in the extracellular compartment. Thus, the percentage of the total enzyme activity in the extracellular compartment fell from 27% in saline control rats to 9% in the endotoxin-treated animals.

Discussion. The present report further

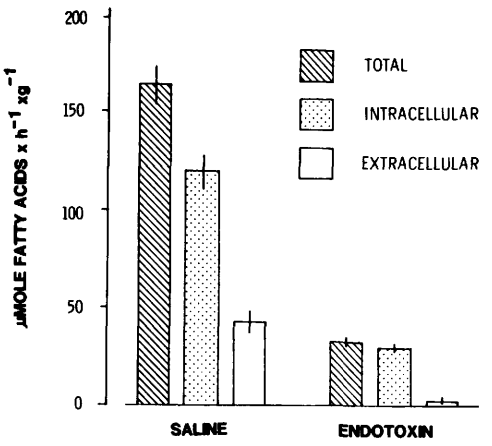

FIG. 2. Total lipoprotein lipase activity in the heart and its distribution between intracellular and extracellular compartments in rats treated with endotoxin or saline. Results are summarized as $\bar{x} \pm$ SE, $N = 7$. Significant differences were present in each value between saline- and endotoxin-treated animals, $P < 0.01$.

supports the proposal of Kaufmann *et al.* (4) that a dysfunction of the triacylglycerol disposal mechanism is important in eliciting the hypertriacylglycerolemic response of endotoxicosis. Tissues normally possessing elevated LPL activities during the fasting conditions of this study, i.e., heart and skeletal muscle of high aerobic capacity, responded to the endotoxin insult by substantially decreasing their activities. The white gastrocnemius muscle was the only skeletal muscle tissue examined which did not respond to the endotoxin treatment with a significant decrease in LPL activity. As reported by others (7), muscles which contain a high percentage of FG fibers, like the white gastrocnemius, possess extremely low activities of this enzyme. This makes it difficult to discern differences if they exist. In any event, it is extremely unlikely that even a complete loss of this activity in this muscle type would have a demonstrable effect on overall handling of circulating triacylglycerol.

The lack of a significant response in adipose tissue to the endotoxin insult has been observed previously (6). Normally adipose tissue LPL activity is low when animals are in the fasted state (5). Thus, one reason a significant decrease was not observed in the adipose tissue of endotoxin-treated rats may have resulted from the already reduced levels produced by using fasted rats.

The widespread decrease throughout the tissues of the endotoxemic rat indicates that the capacity to dispose of VLDL-triacylglycerol is impaired. This effect may be more pronounced if one considers that only extracellular LPL associated with the luminal surfaces of the tissue's capillary endothelial cells comes in contact with circulating triacylglycerol (5). Short-term heparin perfusion of isolated tissues, like the heart, have been used by several investigators to separate and measure the distribution of lipoprotein lipase activity between the intracellular and extracellular compartments (10–12). Although following endotoxin both intra- and extracellular compartments of myocardial LPL activities were depressed, the decrease in extracellular activity was most marked. Other investigators have demonstrated that alterations in heart and adipose tissue activities due to nutritional changes are primarily the result of fluctuations in activity associated with the extracellular compartment (13, 14). Although similar studies have not been performed on skeletal muscle, the ability of this tissue to take up circulating triacylglycerol fatty acids appears to be related to its concurrent level of activity (13). Thus, the differences in enzyme activities between saline- and endotoxin-treated rats seen in tissues besides the heart may be even more pronounced if one considers the functional extracellular compartment separately.

This work was supported by Office of Naval Research Contract N-0014-76-C0133 and Public Health Service Grant GM-7029.

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Received April 13, 1981. P.S.E.B.M. 1981, Vol. 168.