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PULMONARY MACROPHAGE: A MAJOR SOURCE OF LIPOPROTEIN LIPASE IN THE LUNG

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SUMMARY: The lipase released by heparin infusion into perfused rat lungs is shown to be identical with lipoprotein lipase on the basis of the following characteristics: 1) it required plasma for full activity; 2) it was largely inhibited by 1 M NaCl; and 3) it bound to a heparin-Sepharose gel and eluted with buffer containing 1.5 M NaCl. Macrophages prepared from rat lungs released a considerable amount of lipoprotein lipase, with characteristics similar to those of the lipoprotein lipase released from heparin-perfused rat lungs. Pulmonary surfactant-producing type II pneumocytes did not cause a significant release of lipoprotein lipase. No apparent lipoprotein lipase activities were detected in the conditioned medium of other lung cells from which macrophages had been selectively removed.

Uptake of plasma fatty acids by tissue is mediated by lipoprotein lipase (LPL)(1) and previous reports have suggested that LPL is present in lung tissues (2,3). Perfusion of lung with heparin is known to cause the release of LPL (3). It is also known that type II pneumocytes utilize triglyceride fatty acids and synthesize pulmonary surfactant. Probably, LPL in the lung plays a role in supplying such pneumocytes with fatty acids for surfactant synthesis (4). However, little is known about the cellular source of LPL in the lung. In the present study, we have characterized the lung LPL and identified the cells that release the enzyme.

MATERIALS AND METHODS

Lung perfusion: We used male Wistar rats (250-300 g). They were anesthesized with sodium pentobarbital (40 mg/kg). We prepared the lungs for in situ perfusion by cutting away the rib cage and then

Abbreviations: LPL; lipoprotein lipase, FBS; fetal bovine serum

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inserting cannulae into the left ventricle and right atrium as described previously (5). We used an artificial perfusion medium consisting of Hanks balanced salt solution gassed with a mixture of 95% O_2:5% CO_2. Flow rate was maintained at 20 ml per minute through the perfusion period.

Isolation of alveolar macrophages, and type II pneumocytes: To collect alveolar macrophages, the lungs were instilled with Hanks solution (five times with 10 ml per lavage), and the fluid from the lavage was saved to isolate macrophages for studies. Cells retrieved were centrifuged at room temperature at 125 g for 10 min. The cell pellet was then suspended at 1 x 10^6 cells/dish (60 x 10 mm) in F-10 medium (6) with 5% fetal bovine serum (FBS), and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. All the macrophages that would adhere had done so by 4 hr. The dishes were washed and incubated in the same medium for one week. The viability of the cells was 92% at the end of the culture period. Type II pneumocytes were isolated by the method of Mason et al (7). Briefly, lungs were instilled with 0.25% trypsin solution (GIBCO, N.Y.) after removal of the final Hanks solution instilled for macrophage preparation described above. After 10 min, lungs were removed and minced finely in F-10 medium with 10% FBS. The pieces and mincing solution were transferred to a final 100 ml bottle. The bottle was shaken for 10 min in a reciprocating water bath at 37°C. The minces and freed cells were decanted through stainless steel mesh. The dispersed cells were purified by differential adhesion (5,7). Nonadherent cells were collected, and the cultures were incubated in F-10 medium with 5% FBS for one week. The cultured cells were 87% type II pneumocytes, and 2% endothelial cells, with the remainder consisting of ciliated cells, fibroblastic cells, macrophages, and unidentified cells. Viability of the cells was 95% at the end of the culture period.

Macrophage-deprived lung cell fractions: Macrophage-deprived lung cell fractions were obtained by the method described previously (5). Briefly, the lungs removed after lavages, were perfused with 0.1% collagenase solution (Worthington, type II), and then minced as finely as possible. The pieces were digested with 0.5% trypsin and 0.5% collagenase solution at 37°C for 30 min. During digestion, the tissue suspension was stirred magnetically. The cell suspension obtained was centrifuged, and the resultant pellet was resuspended, and washed with F-10 with 10% FBS. To remove macrophages, the cells were incubated in F-10 medium with 10% FBS for 4 hr. at 37°C. Nonadherent cells were transferred to plastic dishes and cultured at 37°C for one week. The cells obtained consisted of 22% type II pneumocytes, 37% endothelial cells, 33% fibroblastic cells, and other unidentified cells. Macrophages were less than 0.5%. The cell viability at the end of the culture period was 82%.

Identification of cells: The cells were examined with light and electron microscope as described (5). Differential cell counts were performed by counting 200 cells on Papanicolaou-stained smears under a light macroscope (8), or by counting 200 cells with the electron microscope, or by both procedures. The viability of the cells was judged by trypan blue dye exclusion test.

Heparin-Sepharose affinity chromatography of triglyceride lipase: 7 ml of postheparin perfusate was applied to a 1.0 x 2.3 cm column of Sepharose containing covalently linked heparin equilibrated with 0.5 mM veronal/HCl buffer containing 0.4 M NaCl (pH 7.4). Elution was carried out using 12 ml of 0.7 M NaCl buffer and 10 ml of 1.5 M NaCl buffer. Each eluate fraction (2 ml) was assayed for enzyme activity. The recovery of lipoprotein lipase from the column was usually 70%.

Assay of lipoprotein lipase: The enzyme activity was determined by the radioisotope method described previously (9). The substrate was a mixture of 2 μCi of glycerol tri[1-^{14}C]oleate (Amersham/Seale Corp.), 0.133 g of unlabelled triolen (Sigma Chem. Co.), 0.9 ml of 4% bovine serum albumin solution adjusted to pH 8.6, 0.9 ml of 1% Triton X-100 and 10.2 ml of Tris-HCl buffer (pH 8.6). The mixture was sonicated on ice for 3 min with Tomy UP-sonifier (Tomy Seiko, Tokyo). To 0.4 ml of this substrate, 0.1 ml of pooled plasma as an activator for lipoprotein lipase and 0.3 ml of 4% bovine serum albumin/0.2 M Tris-HCl buffer (pH 7.4) were added. Incubation was started with the addition of 0.2 ml of samples, and was carried out for 30 min at 37°C. The free fatty acid released during incubation was extracted and the radioactivity in free fatty acid was determined. The enmzyme activity was expressed as nmol free fatty acid/min per ml.

RESULTS AND DISCUSSION

After a single injection of heparin (1000 units) into pulmonary cannula, the perfusion solution was collected from the left ventricular cannula in five tubes at 45-second-intervals. As shown in Fig. 1, the enzyme activities increased immediately after injection and were then rapidly decreased in a few minutes. The amount of lipoprotein lipase activity released within 45 seconds of heparin injection was 22.3 nmol free fatty acid/min. Venous effluent collected after heparin infusion was characterized with the use of a heparin-Sepharose affinity

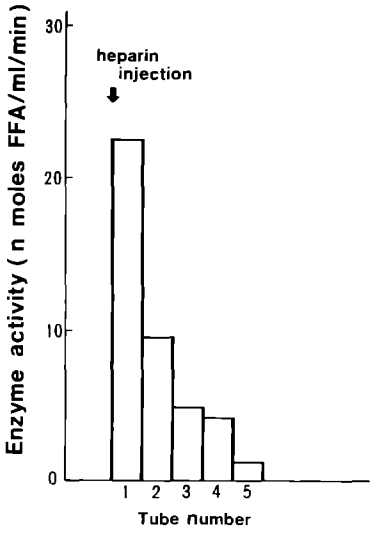

Figure 1. Release of lipoprotein lipase in perfused lungs. After a single injection of heparin (1000 units), the perfusate was collected every 45 seconds and assayed for lipoprotein lipase activity.

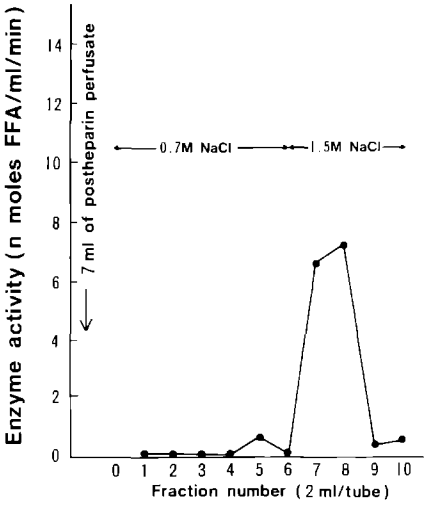

Figure 2. Heparin-Sepharose affinity chromatography of the lung triglyceride lipase. Seven ml of postheparin perfusate was applied to a 1.0 x 2.3 cm Sepharose column covalently linked heparin. A stepwise elution was carried out using 12 ml of 0.7 M NaCl buffer and 1.5 M NaCl buffer. Each fraction (2 ml) was assayed for enzyme activity.

column. While no lipase activity was eluted with the buffer containing 0.7 M NaCl, approximately 70% of the applied lipase activity was eluted with the buffer containing 1.5 M NaCl (Fig. 2). The activity in this column-purified preparation, which had an alkaline pH optimum (pH 8.2), was inhibited by 1 M NaCl and decreased by omission of plasma from the reaction mixture (Table 1).

To determine the cellular sources of lipoprotein lipase in the lungs, different types of cells constituting rat lungs were prepared and incubated *in vitro*. In the conditioned medium of alveolar macrophages, a considerable amount of lipoprotein lipase activity appeared (Table 2). This enzyme activity was inhibited 63% by 1 M NaCl, and 78.4% by protamine sulfate, and exhibited a pH optimum of 8.2. In the absence of plasma, the enzyme activity was reduced by 91%. These

Table 1. Properties of column-purified triglyceride lipase of lungs

Assay condition	Activity (%)
Complete system	100
Serum-omitted	15
Plus 1 M NaCl	33

Table 2. Release of lipoprotein lipase by pulmonary macrophages

Cell type	LPL activity in the medium (nmoles FFA/min/10^6 cells)	LPL activity 30 min after addition of heparin
Macrophages	60.5	16.0
Type II cells	1.3	1.1
Macrophage-deprived lung cell fraction	1.3	1.4

properties are those of lipoprotein lipase. Type II pneumocytes were prepared and incubated in vitro. No significant activities of lipoprotein lipase were detected in the conditioned medium (Table 2). The cells did not release lipoprotein lipase even after the addition of heparin. To determine if the other lung cells contribute to the production of lipoprotein lipase, mixed cell populations were prepared by digesting perfused rat lungs. To exclude the contribution of contaminating macrophages to the release of lipoprotein lipase in mixed cell populations, macrophages were removed by selective adhesion (5). The nonadherent cells were used for this study. These macrophage-deprived lung cell fractions did not release lipoprotein lipase significantly (Table 2), even after exposure to heparin.

The results suggest that the triglyceride lipase activities released into postheparin lung perfusate and released from macrophage share the classical characteristics of lipoprotein lipase, and that the main cellular source of the enzyme in the lung is not the type II pneumocyte, interstitial cells, or endothelial cells, but is the pulmonary macrophages, which release this enzyme spontaneously. Trace of activities found in the conditioned media of type II pneumocytes, or the macrophage-deprived lung cell fractions, may be attributable to the contaminating macrophages (Materials and Methods).

The alveoli in the lung are lined with a surface active material (surfactant) consisting mainly of dipalmitoyl-lecithin (10). The main source of palmitic acid for surfactant synthesis in lungs is plasma fatty acids, and triglyceride fatty acids (11,12). The possible role

of plasma triglyceride fatty acids as a supplier of substrate for surfactant synthesis has been suggested by Felts who reported the uptake of very-low-density lipoprotein triglyceride fatty acids by rabbit lung slices (13). The uptake of plasma triglyceride by tissues is directly related to the level of lipoprotein lipase activity (2). It is possible that lipoprotein lipase released by pulmonary macrophages is transferred to the luminal surface of vascular endothelial cells to hydrolyse circulating triglyceride into fatty acids, and the resultant fatty acid may be transferred to type II pneumocytes for the synthesis of pulmonary surfactant.

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