Localization of lipoprotein lipase mRNA in selected rat tissues

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Abstract Measurements of enzymatic activity have demonstrated that lipoprotein lipase (LPL), the principal enzyme responsible for hydrolysis of circulating triglyceride, is present in a number of tissues including brain, kidney, and adrenal gland. To determine the sites of synthesis of LPL in these tissues, in situ hybridization studies were performed using a non-sense ^{35}S-labeled RNA probe produced from a 624-bp mouse LPL cDNA fragment. Control studies were performed with a sense RNA strand. Using 5-10-μm sections of 5-day-old rat brain, strong hybridization was found in pyramidal neurons of the hippocampus. Positive hybridization, indicating the presence of LPL mRNA, was also found in brain cortex and in the intermediate lobe of adult rat pituitary gland. Specific areas of adrenal and kidney medulla showed hybridization with the probe. LPL mRNA is, therefore, present in a number of specific regions of the body. LPL in these areas may not be important in regulating circulating levels of lipoproteins, but may be essential for cellular uptake, binding, and transfer of free fatty acids or other lipophilic substances. —**Goldberg, I. J., D. R. Soprano, M. L. Wyatt, T. M. Vanni, T.G. Kirchgessner, and M. C. Schotz.** Localization of lipoprotein lipase mRNA in selected rat tissues. *J. Lipid Res.* 1989. **30:** 1569-1577.

Supplementary key words brain • hippocampus • adrenal • kidney • in situ hybridization • triglyceride • free fatty acids.

Lipoprotein lipase (LPL) is the major enzyme responsible for hydrolysis of triglyceride in chylomicrons and the initiation of catabolism of very low density lipoproteins (VLDL) (1-3). The enzyme is secreted by fat and muscle cells, and binds to the luminal surface of extrahepatic capillary endothelial cells where it is available to interact with circulating lipoproteins. In this location, LPL hydrolyzes triglyceride transported in chylomicrons and VLDL, liberating free fatty acids which are used for energy requirements and/or for storage in muscle and adipose tissue. LPL activity in postheparin plasma is inversely correlated with the magnitude of postprandial lipidemia and positively correlated with circulating levels of high density lipoproteins in humans (4, 5). Thus, LPL may be an important factor in regulating blood levels of some atherogenic and anti-atherogenic lipoproteins. LPL activity in adipose tissue is a major determinant of the amount of fatty acid taken up by that tissue. Thus, higher levels of adipose LPL may result in greater fat storage and contribute to or cause obesity in some animals and humans (6, 7). The activity of LPL in muscle and fat is differentially regulated such that with food intake the activity in the fat increases while that in muscle decreases. Thus, LPL plays a role in the distribution of caloric substrates, circulating as triglyceride, to different tissues for oxidation or storage. The increased LPL activity in adipose tissue which occurs with food intake may result from the increased flux of glucose into adipose tissue associated with insulin action (8, 9).

LPL activity in tissues other than fat and muscle may also be responsible for the generation of free fatty acids from circulating triglyceride. These fatty acids could be utilized for cellular energy or lipid biosynthesis. In the brain an additional role for fatty acids as a satiety signal has been postulated. Eckel and Robbins (10) showed that hypothalamic LPL activity in the rat is decreased by prolonged starvation, and they have suggested that local concentrations of free fatty acids produced by LPL in the hypothalamus may be involved in appetite regulation. If correct, brain LPL may be a critical link between fat circulating in the bloodstream and the regulation of food intake. Chajek, Stein, and Stein (11) demonstrated that LPL activity in the brains of rat pups rapidly rises after birth. Brain LPL activity then decreases as the rats mature. Thus, an alternative hypothesis for the role of LPL in the brain is that LPL is required for lipid accumulation during development of this organ.

Abbreviations: LPL, lipoprotein lipase; VLDL very low density lipoproteins; MOPS, morpholinopropanesulfonic acid; PBS, phosphate-buffered saline; SSC, sodium chloride-sodium citrate buffer.

An understanding of the role of this enzyme in the brain and other areas of the body requires fundamental information regarding the tissues and cells responsible for LPL synthesis. Therefore, using in situ hybridization, the sites of production of LPL mRNA were studied in several organs that are known to contain LPL activity. In this report we demonstrate LPL mRNA in neurons of the rat hippocampus and, in less abundance, in the cortex. In addition, LPL mRNA is present in both the adrenal medulla and renal medulla.

METHODS

Northern blot analysis of brain RNA

Tissue preparation. Protocols for these studies were reviewed and approved by the Institutional Animal Care and Use Committee of the Columbia University Health Sciences Division. Female cynomolgus monkeys were killed by phenobarbital injection. Nonfasting adult Sprague-Dawley rats were anesthetized with isoflurane inhalation and decapitated by guillotine. Brains were immediately removed from the skull and areas to be used for mRNA were frozen in liquid N_2.

RNA from rat and monkey brains was prepared by the method of Tushinski et al. (12). Poly (A +)RNA was isolated using oligo(dT)-cellulose affinity chromatography (13). RNA was quantitated spectrophotometrically by absorption at 260 nm. RNA samples for Northern blot analysis were heated for 15 min at 55°C in 50% formamide and 1% MOPS buffer {20 mM morpholinopropanesulfonic acid (pH 7.0), 5 mM sodium acetate, and 1 mM ethelenediaminetetraacetate}. RNA (20 μg) or poly(A +)RNA (10 μg) was used for electrophoresis for 16 h at 40 volts in 1% agarose gel with a running buffer containing 2.2 M formaldehyde and 1 × MOPS. To determine the relative positions of the electrophoresed RNA, a control lane containing liver total RNA was removed from the gel and the positions of the ribosomal RNA bands were determined using ethidium bromide. The RNA in the remainder of the gel was transferred to nitrocellulose paper by osmosis (14), and the paper was dried in a vacuum oven. Mouse LPL cDNA (clone ML5) (15) was labeled with [^{32}P]dCTP (New England Nuclear, Boston, MA) using oligonucleotide labeling (Pharmacia, Piscataway, NJ). The filters were prehybridized with salmon sperm DNA, hybridized overnight with cDNA, and washed with three changes of 2 × SSC (SSC = 0.15 M NaCl, 0.015 M Na citrate, pH 7.0) at 27°C and 1 × SSC with 0.1% sodium dodecyl sulfate at 50°C (16). The filters were placed in cassettes with intensifying screens and exposed to Kodak SB5 X-ray film for 3–5 days at −70°C.

In situ hybridization for LPL mRNA

Preparation of tissues. In situ hybridization studies for LPL mRNA were performed using brains from 5-day-old rats. Because of the small size of the neonatal rat pituitary, adult pituitary glands were used. Also kidneys and adrenal glands from adult animals were used.

Rats were anesthetized by intraperitoneal injection of ketamine (100 mg/kg body weight). The animals were perfused with cold saline followed by 40 ml of PBS containing 4% paraformaldehyde, via a catheter in the left ventricle of the heart and a second catheter for outflow in the right atrium. The organs were removed, immersed overnight in 4% paraformaldehyde, and then in PBS containing 15% sucrose at 4°C until the tissue submerged (1–3 h). The tissues were embedded in OCT compound (Miles Laboratory, Naperville, IL) and frozen in isopentane cooled in liquid nitrogen. Serial 5–10 μm sections were prepared using a cryostat (−20°C) and mounted on poly-L-lysin coated slides. The slides were immediately frozen at −70°C in slide boxes containing desiccant capsules.

Preparation of cRNA probes. The mouse LPL cDNA clone was cut using BAM H1, and a 624-bp fragment containing bases 643–1266 was inserted into a p-Gem 4 vector (Promega Biotec, Madison, WI). The cDNA was amplified and isolated. One μg of linearized LPL cDNA was used in the transcription reaction to provide ^{35}S-labeled RNA LPL probes in the sense and anti-sense orientation. The reaction mixture (10 μl) included 100 μCi [^{35}S]UTP (sp act 1000–1400 Ci/mmol, New England Nuclear), 100 μM UTP, and 500 μM each of ATP, CTP, and GTP, 15 U RNAsin (Promega), 10 μM dithiothreitol, 40 mM Tris, pH 7.5 6 mM $MgCl_2$, 2 mM spermidine, and 15 U of either SP6 or T7 polymerase (Boehringer Mannheim, Indianapolis, IN). After 1 h at 37°C, 100 μl of DNAse buffer was added (400 mM Tris, pH 8.0, 100 mM NaCl, 60 mM $MgCl_2$) and the DNA was digested with 3 units of DNAse (RQI DNAse, Promega) at 37°C for 20 min. After phenol extraction and ethanol precipitation, integrity of the probes was assessed by polyacrylamide gel electrophoresis. Probes were stored at −70°C for up to 3 weeks.

In situ hybridization technique. In situ hybridization of serial sections of the entire 5-day-old rat brain, adult pituitary, and multiple sections of kidney and adrenal glands was performed using a modification of the Wilcox, Gee, and Roberts method (17). The slides were removed from the freezer and immediately covered with proteinase K (1 μg/ml) in 20 mM Tris-HCl (pH 7.5), 2 mM $CaCl_2$ for 10 min at room temperature, washed with sterile water and then immersed in 0.1 M triethanolamine (5 min) and 0.25% acetic anhydride in 0.1 M triethanolamine (10 min) at room temperature. They were washed in 2 × SSC, prehy-

bridized for 2 h at 55°C in a solution containing 0.5 mg/ml salmon sperm DNA, 50% formamide, 600 mM NaCl, 10 mM Tris, pH 7.5, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.1% bovine serum albumin, 1 mM ethylenediaminetetraacetate, 0.5 mg/ml total yeast RNA, and 50 μg/ml yeast transfer RNA. Hybridization occurred overnight at 55°C under the same conditions but included 10 mM dithiothreitol, 10% dextran sulfate, and 2,500 cpm/μl [^{35}S]UTP RNA probe. The slides were washed in 2 × SSC, digested with RNAse A (20 μg/ml) and RNAse T1 (2.2 μg/ml) (Sigma Chemical Co., St. Louis, MO) for 45 min at 37°C, and washed again in 2 × SSC and 0.1 × SSC containing 14 mM β-mercaptoethanol and 0.05% sodium pyrophosphate at 53°C. The slides were placed in washing solution at room temperature overnight, dehydrated in ethanol, vacuum dried, and exposed to photographic film emulsion (NTB2 nuclear track emulsion, Eastman-Kodak, Rochester, NY) for 4–6 weeks at 4°C, developed, fixed, and then stained with hematoxylin and eosin. Control slides included those processed without the probe and others processed using the nonhybridizing sense RNA strand.

RESULTS

A Northern blot of mRNA obtained from rat and monkey brains is shown in **Fig. 1**. Using pooled rat brain RNA a single faint band hybridizing with LPL cDNA was found at 3.6 Kb. This band was at a position similar to one found with RNA from rat epidydimal fat. Similarly, a weak signal for LPL in mouse brain has been reported (15). A stronger signal was noted using monkey RNA obtained from several areas of the cerebral cortex, cerebellum, or brainstem. Two transcripts, 3.6 and 3.4 Kb, were found using monkey mRNA and were similar in size to those found with human tissue. Wion et al. (18) have shown that these two transcripts are due to alternative polyadenylation sites.

In situ hybridization for LPL mRNA was performed using coronal sections of the rat brains. An intense signal, indicating the presence of LPL mRNA, was found in the hippocampus, and a weaker but detectable signal was present in the cortex. As seen under dark field illumination (**Fig. 2**), LPL mRNA was localized to these structures. High power magnification of the hippocampus photographed using bright field illumination is shown in **Fig. 3**. The silver grains demonstrating hybridization are seen overlying pyramidal neurons. These cells can be identified by the presence of the characteristic clear, nonstaining areas. No obvious signal was found associated with glial elements. Glial cells are the predominant cellular elements of brain. Thus, the expression of LPL gene predominantly in neurons explains the relatively weak signals found when rat (see above) and mouse brain RNA (15) were probed for LPL mRNA using Northern blot analysis. However, this may not explain the apparent difference in LPL mRNA levels between the monkey and rodent brains.

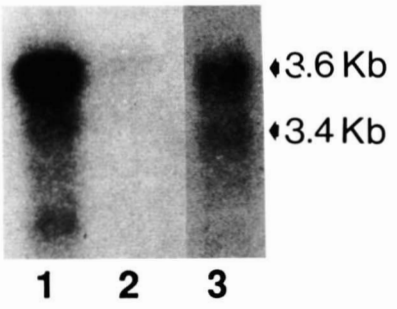

Fig. 1. Northern blot analysis of brain LPL mRNA. Ten μg of poly (A +) RNA from rat epidydimal fat (1), rat brain (2), and monkey brain (3) were used for electrophoresis in a 1% agarose gel, transferred to nitrocellulose, and hybridized with ^{32}P-labeled mouse LPL cDNA as described in Methods. The darkest band seen in lanes 1 and 3 and seen faintly in lane 2 is at 3.6 Kb.

An adult rat pituitary gland was fixed and sectioned to detect LPL mRNA. Because previous studies had shown no signal for LPL mRNA in this region by Northern blot analysis (data not shown), the slides were allowed to develop for 6 weeks. In **Fig. 4** are photographs of sections of pituitary hybridized with anti-sense (specific, Fig. 4a) and sense (nonspecific, Fig. 4b) cRNA probes. Although a significant amount of background was noted, no specific hybridization was found in either the anterior or posterior pituitary gland. However, specific hybridization was found in the intermediate lobe of the pituitary suggesting that this region contained LPL mRNA.

In situ hybridization studies for LPL mRNA in adult rat kidney are shown in **Fig. 5**. An intense signal, as expected, was found in perinephric fat, and no specific signal was found in renal cortex (Fig. 5a). However, within the renal medulla, an area corresponding to the outer zone contained LPL mRNA. This can be seen in the longitudinal section shown in Fig. 5b. This region having positive hybridization for LPL mRNA contains the thick ascending limb of the distal tubule. The mRNA was localized to the tubular epithelial cells. Only background levels of staining were seen in the inner medulla.

In situ hybridization for LPL mRNA was also performed using sections of adult rat adrenal gland. A comparison of photomicrographs of studies using anti-sense (**Fig. 6a**) and control sense probes (Fig. 6b) readily demonstrates that specific intense hybridization occurred in cells of the adrenal medulla. No specific hybridization was found in the adrenal cortex. A higher magnification (Fig. 6c) demonstrated that the LPL mRNA was in the area of the adrenal medulla which is predominantly comprised of chromaffin cells.

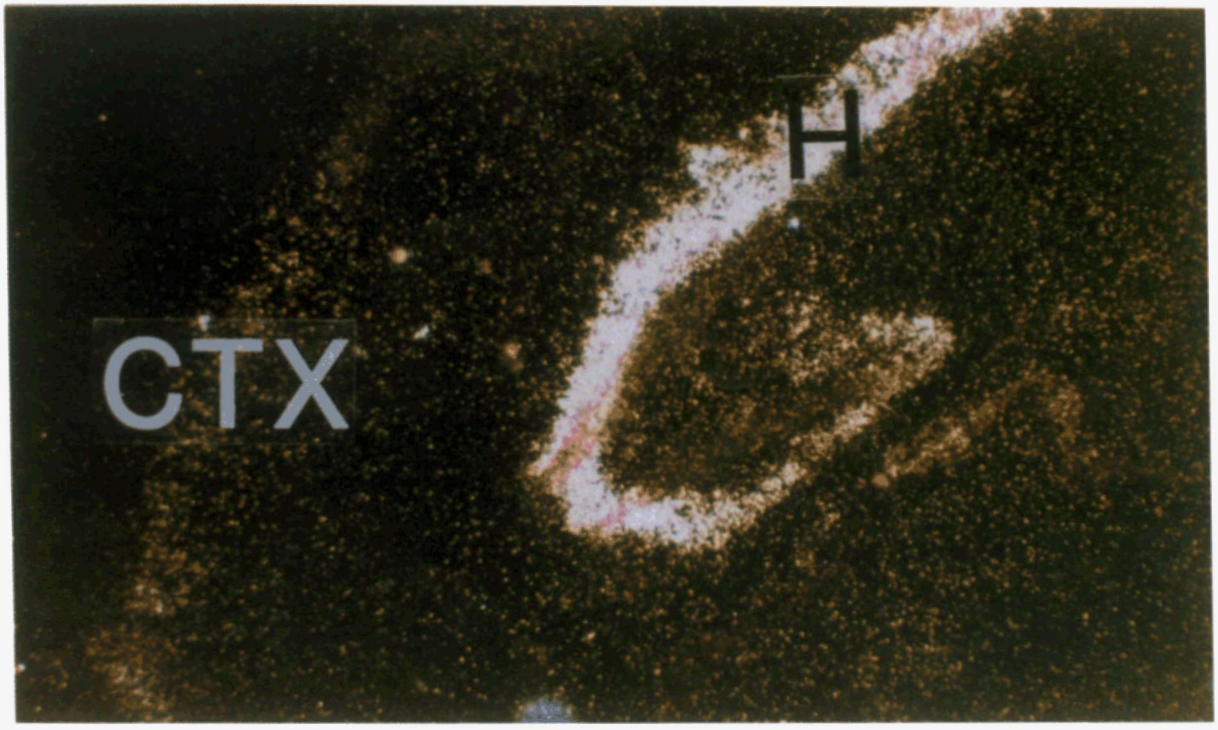

Fig. 2. In situ hybridization to localize LPL mRNA in the bain from a 5-day-old rat. The photomicrograph is of a coronal section of brain that was hybridized with ^{35}S-labeled anti-message-sense LPL cRNA and exposed for 4 weeks. The photographs were taken using polarized light epiluminescence so that the silver grains appear white. The galaxy-like structure showing intense hybridization is the hippocampus (H), less dramatic hybridization is also apparent in the cortex (CTX), × 40.

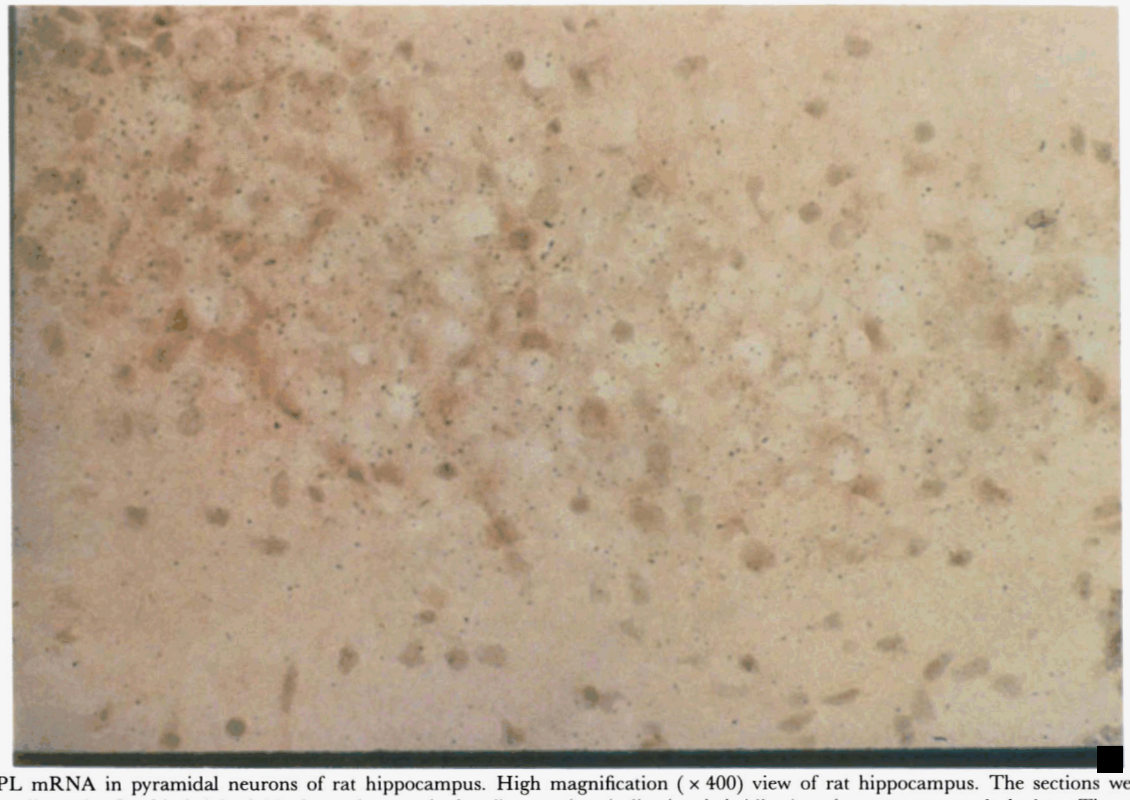

Fig. 3. LPL mRNA in pyramidal neurons of rat hippocampus. High magnification (× 400) view of rat hippocampus. The sections were stained with hematoxylin-eosin. In this bright-field photomicrograph the silver grains, indicating hybridized probe, are seen as dark dots. The cells which have characteristic clear areas are pyramidal neurons.

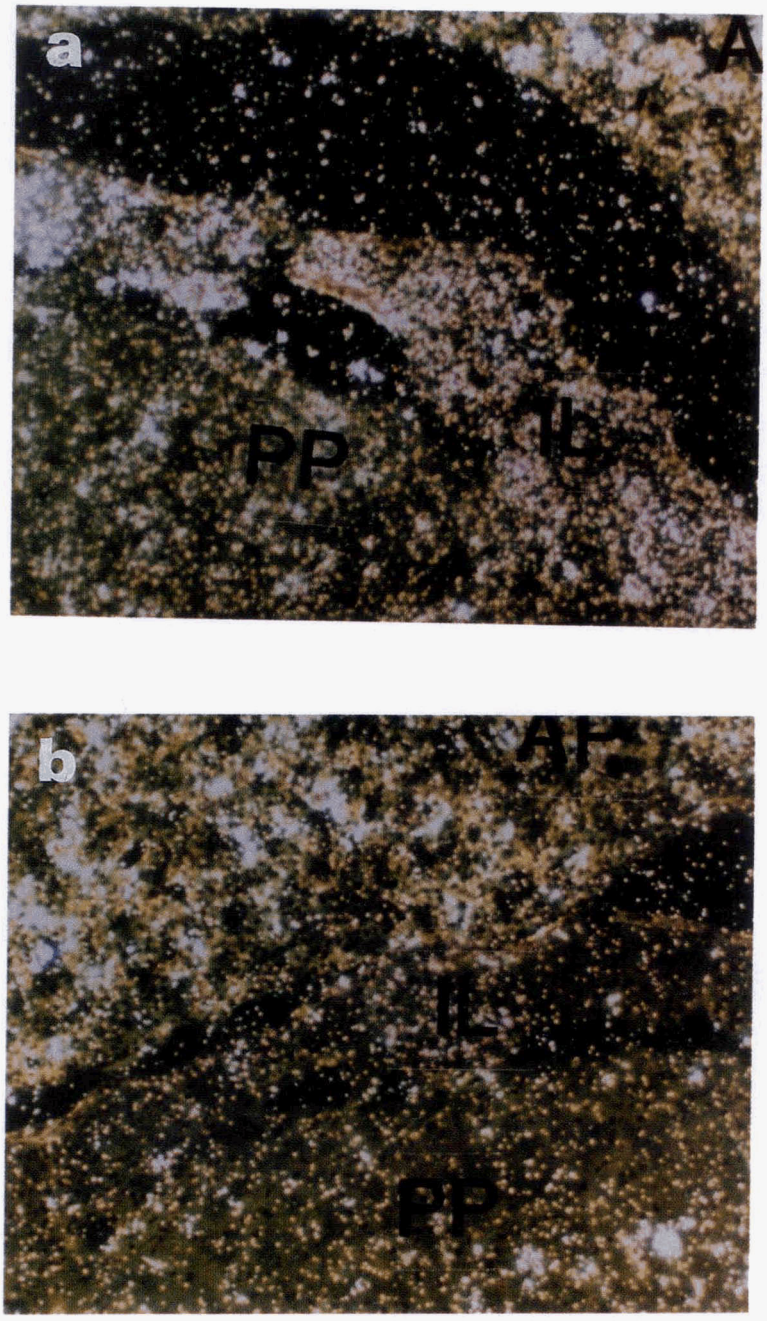

Fig. 4. In situ hybridization of adult rat pituitary to ^{35}S-labeled LPL anti-message-sense (a) and sense (b) cRNA probe. The white dots, visualized by polarized light epiluminescence, are seen in high concentration in the intermediate lobe (IL). The staining in the intermediate lobe was not found using control sense RNA probe. Background staining but no specific hybridization was found in the posterior pituitary (PP) or anterior pituitary (AP). Exposure 6 weeks, magnification × 100.

DISCUSSION

Our studies demonstrate that LPL mRNA is localized to the cortex and hippocampus of the brain of rat pups. In addition, LPL mRNA was found in adult pituitary gland intermediate lobe, adreanal medulla, and renal medulla. Although LPL-like activity has been previously reported in rat brain (10, 11), using a specific inhibitory monoclonal antibody (19), we had previously established that the enzyme in monkey brain was, indeed, LPL and not another triglyceride lipase active at neutral pH (20). LPL is a secreted protein, which binds to glycosaminoglycans on capillary endothelium, and is found, albeit at low levels of activity, circulating in the bloodstream (21,22). Theoretically, some of

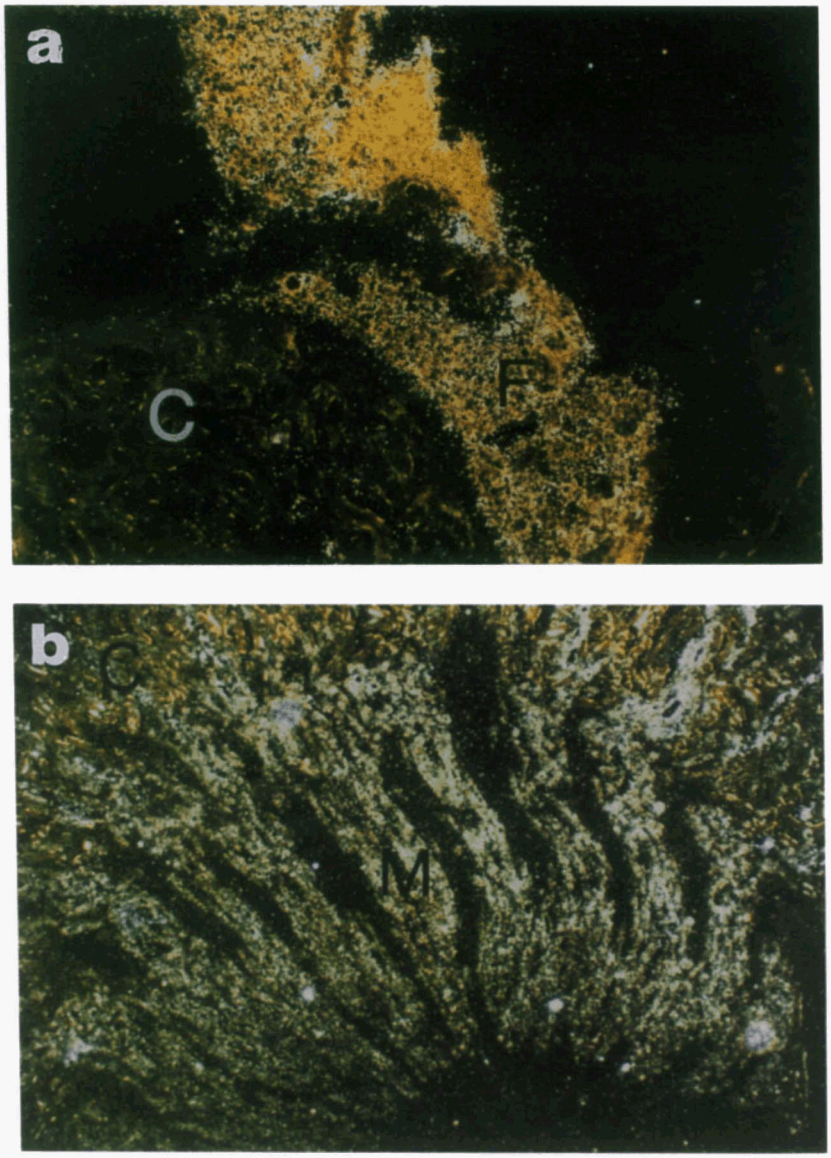

Fig. 5. In situ hybridization of adult rat kidney for LPL mRNA. Sections of kidney from adult rats were prepared as described in Methods and hybridized with ^{35}S-labeled LPL anti-message-sense cRNA. The photographs (× 100) were taken using polarized light epiluminescence so that the silver grains appear white. In Fig. 5a, no signal was found in the renal cortex (C) but intense signal, indicating an abundance of LPL mRNA, was seen in perinephric fat (F). Fig. 5b shows hybridization in a specific area of renal medulla (M), corresponding to the distal tubule.

this circulating enzyme could attach to endothelial surfaces throughout the body. Brecher and Kuan (23) had reported that preparations of brain microvasculature hydrolyze triglyceride. This finding suggested that LPL, either synthesized locally or synthesized in other tissues and transported to the brain via the bloodstream, is present on the brain endothelium. Because LPL mRNA is found in the brain, some of the LPL activity found there is likely to be produced locally.

The levels of LPL activity in the rat brain vary widely during development, with the highest levels reported at approximately 4 days (11). For this reason, we initially chose to localize brain LPL mRNA in the rat pup. The dramatic hybridization found in neurons of rat hippocampus was somewhat unexpected. The neuronal site of LPL gene expression, shown in our studies, suggests that LPL may be produced in areas which are not likely to directly interact with circulating lipoproteins. Aside from hydrolyzing triglyceride, LPL, in vitro, functions as a transfer protein for cholesteryl esters (24) and vitamin E (25). Similar actions

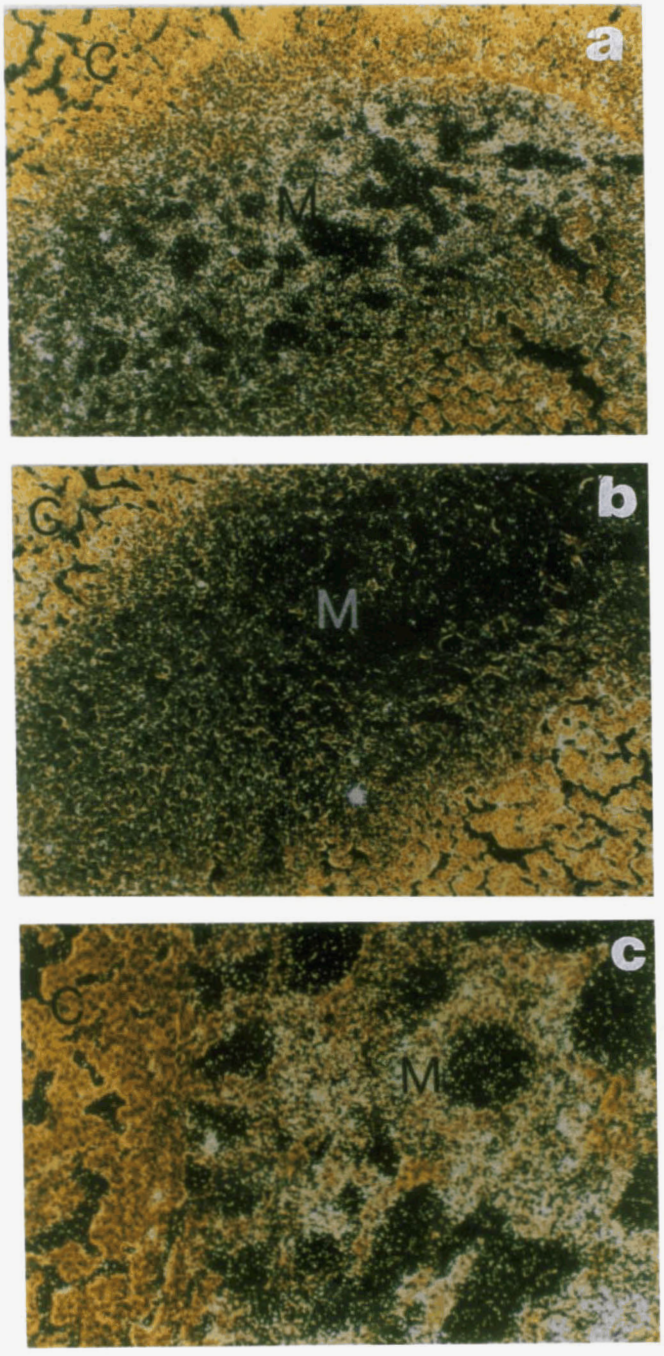

Fig. 6. In situ hybridization of adult rat adrenal gland with ^{35}S-labeled LPL anti-message-sense (a and c) and sense (b) cRNA probe. The white dots, visualized by polarized light epiluminescence, are seen in high concentration in the adrenal medulla (M) with less specific staining in the cortex (C). The staining in the adrenal medulla seen at two magnifications (a = 40 × and C = 100 ×) was not found using control sense RNA probe (b).

involved in lipid transport may be primary roles of LPL in the brain and may be important for normal neurological development or function.

Detectable levels of LPL mRNA were found in the cerebral cortex and the intermediate lobe of the pituitary. Because the anterior pituitary contains a variety of cells that synthesize different peptides and because of the relatively low level of hybridization in this tissue, we cannot be certain that a subpopulation of these cells (e.g., those that produce adrenocorticotrophic hormone) do not contain LPL mRNA. In agreement with our previous findings (20), Rule et al. (26) have reported that LPL activity is higher in rat pituitary than other areas of the brain, and that this activity may be modulated by steroid hormones. Our results demonstrating LPL mRNA in the intermediate lobe of the pituitary suggest that the findings of Rule and co-workers (26) may be due to regulation of LPL production in the intermediate lobe of the pituitary gland. Alternatively, the rich capillary system in the pituitary may allow a greater amount of LPL circulating in the blood (27) to attach to glycosaminoglycans on the surface of pituitary cells. In this regard, Doolittle et al. (28) have recently demonstrated that another triglyceride-hydrolyzing enzyme, hepatic triglyceride lipase (HTGL), which is found in the adrenal gland, is not synthesized in that organ. HTGL is presumably transported in the bloodstream attached to circulating lipoproteins and is taken up by adrenal tissue along with those lipoproteins. Thus, some of the LPL activity in the pituitary and other organs may be synthesized elsewhere. However, such an occurrence would not explain the presence of the mRNA for LPL.

LPL activity and LPL and mRNA have been reported in renal (15,29) and adrenal tissue (15,30). Our data locate the sites in those organs where this enzyme is likely to be synthesized. Limited physiological data have suggested that kidney, like muscle, can produce free fatty acids by hydrolysis of circulating VLDL triglyceride (31). The importance of free fatty acids for energy requirements of the renal medulla, as opposed to cortex, may be quite limited (32). However, LPL actions may be important for functioning of the specific area of the distal tubule where LPL mRNA is found. The role of LPL in adrenal chromaffin tissue also remains to be defined. Because the adrenal medulla is derived from neuroectodermal tissue, it may be that LPL in this area serves a function similar to that in the brain.

Immunohistochemical studies of LPL protein have been difficult to perform. LPL is an unstable molecule and is released from its endothelial cell binding site by heparin or high ionic strength buffers. Immunohistochemical studies have, thus far, been limited to adipose tissue (33), a region especially rich in LPL, or have demonstrated LPL on capillary walls by perfusing tissues with antibodies (34) or have shown LPL protein on cell surfaces, e.g., macrophages (35). An important question that remains to be answered is whether LPL protein is found in highest concentration in the areas in which its mRNA is found. In addition, studies on the developmental changes in LPL mRNA (36) complement and should help define future investigations aimed at elucidating the actions of LPL in brain, adrenal, and kidney. We have also performed in situ studies for LPL mRNA

using several other tissues in which LPL mRNA has been reported. However, because of the high amounts of background staining, definitive localization was not demonstrated. Tissue-specific modifications of the in situ technique will be required for several of these tissues and are currently being investigated.

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