Spinal cord of the rat contains more lipoprotein lipase than other brain regions

Daniel H. Bessesen,[1,§,] Connie L. Richards,[§,**] Jacqueline Etienne,[*] John W. Goers,[†] and Robert H. Eckel[§]**

Laboratoire de Biochimie,[*] Faculte de Medecine St. Antoine, Paris 75012, France; Department of Chemistry,[†] California Polytechnic State University, San Luis Obispo, CA 93407; Division of Endocrinology,[§] Department of Medicine, University of Colorado Health Sciences Center, 4200 E. Ninth Avenue, Denver, CO 80204; and Division of Endocrinology,[**] Denver General Hospital, 777 Bannock Street, Denver, CO 80204

Abstract Lipoprotein lipase (LPL) is important for the delivery of triglyceride fatty acids (TGFA) to a variety of tissues. We have used measurements of heparin-releasable LPL activity, immunohistochemistry, in situ hybridization, and Northern analysis to more fully characterize the location of LPL within the entire central nervous system (CNS) of the rat. Surprisingly, the levels of LPL activity and mRNA in the caudal spinal cord were 5- to 10-times higher than those found in any other area of the brain, levels similar to those found in adipose tissue or skeletal muscle. A number of cell types including neurons in the hippocampus, Purkinje cells of the cerebellum, and cells deep within the cortex were identified as sources of LPL mRNA. LPL protein was found within many vascular structures throughout the CNS, and within Purkinje cells. The strongest immunostaining was around nerve rootlets associated with the caudal spinal cord. Feeding studies were carried out with [^{14}C]oleic acid to see whether CNS LPL functioned in the uptake of TGFA. These studies demonstrated uptake of chylomicron triglyceride fatty acids throughout the CNS. The localization of LPL within these structures suggests that the uptake of triglyceride fatty acids is an integral part of normal lipid metaboism of the central nervous system and may be important in regulating feeding behavior and/or maintaining normal neuronal function.—**Bessesen, D. H., C. L. Richards, J. Etienne, J. W. Goers, and R. H. Eckel.** Spinal cord of the rat contains more lipoprotein lipase than other brain regions. *J. Lipid Res.* 1993. **34:** 229–238.

Supplementary key words neuron • glial cell • dietary fat • brain • triglycerides • hippocampus • Purkinje cells • in situ hybridization • immunohistochemistry

Lipoprotein lipase (LPL) is rate-limiting for the removal of lipoprotein triglyceride fatty acids (TGFA) from the circulation, including long chain dietary fatty acids that initially circulate as chylomicron triglyceride (TG) fatty acids. LPL has been extensively studied in adipose tissue and to a lesser extent in muscle, but is also synthesized by lung, lactating mammary gland, pituitary gland, brown adipose tissue, spleen, ovary, macrophages, kidney, and cardiac muscle (1). A number of groups have identified LPL mRNA and protein within the central nervous system (CNS) (2–7). Previous studies have focused in particular on the synthesis and regulation of LPL in the hippocampus where it appears to be especially abundant (8–10).

Previous studies, however, have not included an examination of the spinal cord and/or have not simultaneously used multiple techniques to corroborate the sites of LPL synthesis, location of the protein, enzymatic activity, and function. Additionally, most previous studies of LPL activity in the CNS measured total extractable enzyme activity, which includes both intracellular and extracellular activity not just the physiologically active endothelial bound compartment. A closer examination of LPL in the CNS would begin to give insights into the role that TGFA play in the normal lipid physiology of the CNS. Additionally, by delivering TGFA to neurons, LPL made in the CNS might be involved in providing information to the brain on the state of TG fuels within the organism.

Therefore LPL mRNA, protein, enzyme activity, and function were assessed along the entire length of the CNS of the rat. LPL mRNA was localized by in situ hybridization, and was quantitated by Northern analysis. LPL protein was localized by immunohistochemistry, and the heparin-releasable fraction of enzyme activity was measured. While containing more than just endothelial bound

Abbreviations: LPL, lipoprotein lipase; ISH, in situ hybridization; TG, triglyceride; FFA, free fatty acid; TGFA, triglyceride-derived fatty acids; EFA, essential fatty acids; CNS, central nervous sytem; KRP, Krebs-Ringer phosphate buffer; PBS, phosphate-buffered saline; Co, cerebral cortex; Ce, cerebellum; Hi, hippocampus; Hy, hypothalamus; Pi, pituitary gland; Cv, cervical spinal cord; Th, thoracic spinal cord; UA, upper abdominal spinal cord; LA, lower abdominal spinal cord; CE, caudal spinal cord and associated nerve rootlets.

[1]To whom correspondence should be addressed at: Mail Code 4000, Division of Endocrinology, Denver General Hospital, 777 Bannock Street, Denver, Co 80204.

LPL, the heparin-releasable compartment is thought to more closely reflect the physiologically active compartment. The function of CNS LPL was assessed by following the metabolic fate of ingested [^{14}C]oleic acid incorporated into chylomicron triglycerides.

METHODS

Animals

Sprague-Dawley rats weighing 140–160 g were obtained from Sasco and housed in the temperature-controlled 12 h–light/12 hr-dark (lights on at 0600) Animal Resource Center at the University of Colorado Health Sciences Center (UCHSC). "Fed" rats had free access to rat chow ad libitum until they were killed at 0800. "Fasted" rats had chow removed at 1500 the afternoon prior to being killed at 0800. Protocols were approved by the Animal Use Committee at UCHSC.

Measurements of LPL activity

Immediately after killing the neuroaxis was removed and pieces of frontal cortex, hippocampus, lateral cerebellar cortex, and the entire hypothalamus were removed and placed in Krebs-Ringer phosphate (KRP) on ice. The entire length of the spinal cord, including nerve rootlets contained within the spinal canal, was removed and divided along its length into five equal pieces, a sample from the center of each piece was placed in KRP on ice. Heparin-releasable LPL activity was measured as previously described (11). Briefly, pieces of tissue (40–50 mg) were incubated in 0.4 μl of KRP with 30 μg/ml heparin at 37°C for 45 min. An aliquot of the supernantant was removed and incubated for 45 min at 37°C with a synthetic substrate containing 4 μCi [1-^{14}C]triolein, unlabeled triolein, human serum, egg lecithin, fatty acid-poor bovine serum albumin, and Tris-HCL as a buffer. LPL activity was expressed as nmol of hydrolyzed ^{14}C-labeled fatty acids per gram of tissue. Additional studies were performed to demonstrate that the lipolytic activity was indeed LPL. These included incubating heparin-released material with a 1:10 dilution of goat anti-rat LPL polyclonal antibody (12) or nonimmune goat serum for 2 h at 4°C prior to adding the substrate, or carrying the hydrolysis reaction out in 2 M NaCl in the absence of apoC-II.

Measurements of LPL mRNA

Total cellular RNA was isolated by a modification of the method of Chomczynski and Sacchi (13). Immediately after the rats were killed fresh specimens of brain and spinal cord were collected as outlined above, and placed into solution D (guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sarcosyl, and 0.1 M beta mercaptoethanol). These tissue samples were homogenized and extracted with phenol and chloroform as described (13). Thirty μg of total cellular RNA from each region of the CNS was loaded onto 1% agarose gels containing 5% formaldehyde and 1× MOPS buffer and 0.008% ethidium bromide. After electrophoresis, gels were photographed under UV illumination to document equivalent loading; RNA was then transferred to nitrocellulose and probed with a ^{32}P-labeled 874-bp cDNA for LPL (14). Blots were washed in 0.1× SSC and 0.1% SDS at 52°C for 20 min, and exposed to X-ray film for 7–10 days. The density of signals seen on the autoradiograms was evaluated with a Bio-Rad 620 video densitometer.

Immunohistochemistry

After the rats were killed, they were perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS). Brain and spinal cord were removed and frozen in 2-methyl butane immersed in liquid nitrogen. Frozen sections (16 μm) were made of the entire length of the central neuroaxis and thawed onto slides coated with triethoxysilane (TES). These sections were blocked with 5% rabbit serum in 1% albumin in PBS at 4°C for 12 h, rinsed with PBS, then incubated with a 1:100 dilution of goat anti-bovine LPL polyclonal antibody (12), or a 1:100 chicken anti-bovine LPL polyclonal antibody (15) in 1% albumin and PBS overnight at 4°C. Sections were then washed for 5 min × 3 with 0.1% Triton in PBS, and incubated with a rhodamine-conjugated F(ab')2 anti-goat or anti-chicken secondary antibody for 3 h at room temperature. Finally, sections were washed with 0.1% Triton in PBS at room temperature for 15 min ×3, coverslipped with aqueous mounting medium, and photographed with an Olympus BH2 fluorescence microscope or a Nikon microscope equipped with Bio-Rad confocal optics.

In situ hybridization

Sections were prepared as outlined above with the exception that slides were prewashed and baked overnight at 200°C. Sections were dehydrated through graded alcohols, then prehybridized at 42°C in a solution containing 50% formamide, 3× SSC, 10 mM Tris (pH 7.4), 1 mM EDTA, 200 μg/μl tRNA, and 1× Denhardts, (preheated to 70°C and quick cooled) for 2 h. The prehybridization solution was tapped off and a hybridization mixture was added with the same components as above with 200 K dpm/60 μl of either sense or anti-sense LPL riboprobe and 10 mM dithiothreitol (DTT) and incubated at 42°C for 16 h. Sense and anti-sense ^{35}S-labeled LPL riboprobes were generated from an 874 pb LPL cDNA clone (14) in a PGEM-7 vector using either T7 or SP6 RNA polymerases. Sections were then washed in 4× SSC with 10 mM DTT at room temperature for 15 min, then in 2× SSC for 30 min, then 0.1× SSC in 0.1% SDS at 50°C for 15 min. Sections were then dehydrated, dipped in Kodak NTB-2 emulsion, and stored at 4°C in light-tight boxes

for 7 days before being developed, counterstained with hematoxylin, mounted, and examined by light or darkfield microscopy.

[^{14}C]oleate feeding studies

Rats that had been fasted for 12 h spontaneously consumed 42 nmol of [1-^{14}C]oleic acid along with 100 μg of unlabeled olive oil on 80 mg of white bread and were killed at 20 min, 1, 2, 4, 8, 24 h, 2, 10, and 30 days (n = 4 at each time point) after ingestion of the labeled fatty acids. After opening the calvarium and spinal canal, thereby removing all external adipose and muscle tissues, the neuroaxis was removed, including the entire brain and spinal cord, and homogenized with a Polytron homogenizer in 3 ml of KRP buffer per gram of tissue. Duplicate 500-μl aliquots of the homogenate were placed in scintillation vials with 1 ml of tissue solubilizer (Solvable; NEN) and placed in a 50°C water bath for 3 h. Finally, 50–100 μl of H_2O_2 was added to discolor the samples (if needed) and 10 ml of Aquasol scintillation cocktail was added to each sample; the ^{14}C activity was determined in a Beckman LS6000TA scintillation counter. For studies of regional uptake, rats were fed 84 nmol of [1-^{14}C] oleic acid (n = 5) and killed 2 h after ingesting the label. The brain regions outlined in the studies of regional LPL activity were removed and assessed for ^{14}C content. All results of ^{14}C uptake were corrected for the background activity present in a specimen containing all components except labeled tissue.

Statistics

Two-way comparisons were analyzed with a Student's *t* test. Comparisons among larger groups were done using an analysis of variance. Data are presented graphically as the arithmetic mean ± SE.

RESULTS

LPL enzyme activity

Fig. 1 demonstrates heparin-releasable LPL activity along the entire neuroaxis in both the fasted and fed states. As can be clearly seen, the caudal spinal cord has the highest levels of LPL activity within the CNS. These are levels of LPL activity that are similar to the usual level of heparin-releasable LPL activity in adipose tissue and skeletal muscle (2–5 and 10–20 nmol FFA/g per h, respectively). The hippocampus had the highest levels of LPL activity found within the brain.

The response to feeding was only statistically significant in the thoracic (P = 0.038) and caudal (P = 0.046) spinal cord. We have also measured LPL activity in rats during postnatal development, and found that from the 4th week of life to the 50th there was a steady decline in fasting levels of LPL within the spinal cord (data not shown). At all ages caudal spinal cord LPL increased with feeding. Spinal cord LPL was lower at postnatal weeks 1 and 2 compared to week 4; however, it was difficult to adequately collect all of the caudal cord and associated nerve rootlets at these time points making the quantification of overall spinal cord activity unreliable.

To demonstrate that the lipolytic activity identified in the spinal cord was indeed LPL, enzymatic activity was measured either in high salt in the absence of apoC-II, or after preincubation of heparin-released proteins with an immunoinhibiting polyclonal antibody. This resulted in greater than 90% reduction in measured activity in the caudal spinal cord. In these experiments mean LPL activity in the caudal spinal cord was 17.1 nmol FFA/g per h. Incubation in high salt in the absence of apoC-II reduced the activity to 0.29, incubation with nonimmune serum reduced activity to 8.52, and incubation with an anti-

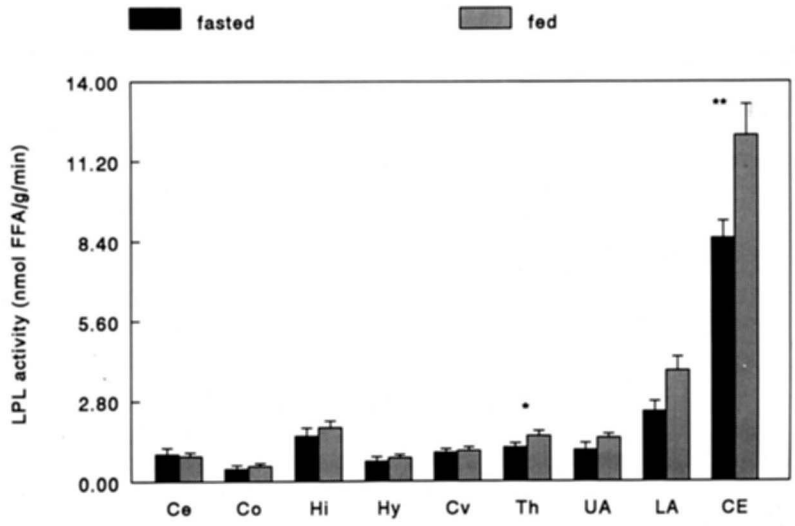

Fig. 1. Heparin-releasable LPL activity (n = 10, ± SEM) in regions of rat brain and spinal cord is depicted in both the 12-h fasted (solid bars) and fed (hatched bars) states. CNS regions are labeled as follows: Ce, cerebellum; Co, cerebral cortex; Hi, hippocampus; Hy, hypothalamus; and the spinal cord regions are listed rostral to caudal: Cv, cervical; Th, thoracic; UA, upper abdominal; LA, lower abdominal; CE, caudal spinal cord and associated nerve rootlets. Significant feeding effects are indicated by * (P=0.038) and ** (P=0.046).

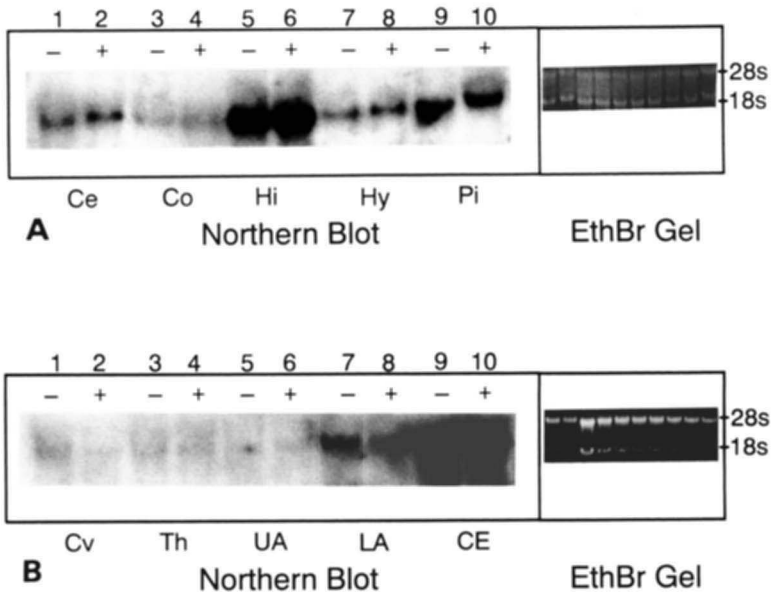

Fig. 2. Northern analysis of LPL mRNA from regions of the rat brain (A) or spinal cord (B). Each lane contains 30 μg of total cellular RNA electrophoresed in a 1% agarose gel, transferred to nitrocellulose, and probed with an 874-base pair LPL cDNA. Brain regions are labeled as follows: Ce, cerebellum; Co, cerebral cortex; Hi, hippocampus; Hy, hypothalamus; and Pi, pituitary of either 12-h fasted (–) or ad libitum fed (+) rats. The five spinal cord regions are labeled as follows: Cv, cervical; Th, thoracic; UA, upper abdominal; LA, lower abdominal; CE, caudal spinal cord and associated nerve rootlets. To document equivalent loading. photographs of the ethidium-stained gels are also shown.

LPL antibody as described in the Methods section resulted in a reduction in activity to 0.8 nmol FFA/g per hr.

LPL mRNA by Northern analysis

Northern analysis of the LPL mRNA content of various brain regions is shown in **Fig. 2A.** LPL mRNA was found in the hypothalamus, cerebral cortex, cerebellar cortex, and pituitary gland, but the most abundant source of LPL mRNA in the brain was the hippocampus. The content of LPL mRNA within regions of the spinal cord in both the fasted and fed states is shown in Fig. 2B. To demonstrate equivalent loading, the contents of 18 and 28S RNAs in the same samples are shown in the accompanying photographs of the ethidium bromide-stained gels. It is clear that LPL mRNA increases caudally just as enzyme activity does. Densitometry of the LPL bands indicates that LPL mRNA is 5.4 times more abundant in the caudal spinal cord when compared to the cervical cord. There was no significant change in LPL mRNA with feeding in any region of the CNS.

Immunohistochemistry

In other tissues LPL functions at the capillary endothelial surface adjacent to the cell of origin. It was no surprise then that there was strong labeling of many vascular structures within the CNS (**Fig. 3A**). In addition, there was staining of cell bodies within the hippocampus and within Purkinje cells and their dendritic extensions within the cerebellar cortex (Fig. 3B, C). In the spinal cord most of the staining was in the gray matter and vascular structures (Fig. 3D). In the caudal spinal cord there was strong immunostaining of all nerve rootlets; however, some stained more than others (Fig. 3E). Within individual nerve bundles there was strong staining around nerve fibers with lighter staining within axons themselves (Fig. 3F). Other regions of the brain and brainstem stained less strongly. An equivalent exposure of an adjacent section stained with nonimmune control serum showed minimal signal (not shown).

In situ hybridization

LPL mRNA was identified in cells deep in the cerebral cortex (**Fig. 4A, B**). The strongest staining for LPL mRNA in the brain, however, was in the hippocampus (Fig. 4C, D) and the Purkinje cell layer of the cerebellum (Fig. 4E). Surprisingly little signal was observed in the hypothalamus despite strong immunostaining for LPL protein in the nearby basal large blood vessels. In the spinal cord the strongest labeling was in the white matter, although a few large cell bodies within the gray matter also were labeled (Fig. 4F). Adjacent sections in each of

these anatomic regions had minimal or no hybridization when incubated with an equal amount of a sense riboprobe (not shown).

^{14}C feeding studies

The time course of ^{14}C uptake within the entire CNS is shown in **Fig. 5.** In these studies rats were fed 42 nmol of 1-^{14}C-labeled oleic acid. The CNS reaches a peak in ^{14}C uptake at 2 h temporally coincident with the peak of plasma ^{14}C activity. There was a 30% decrease in activity over the next 6 h. The activity fell more gradually over the next 1.5 days, and was relatively stable over the next 28 days.

Fig. 6 demonstrates the regional uptake of labeled dietary fat expressed per gram of tissue to indicate the relative abilities of these different regions of the CNS to take up triglyceride fatty acids. In these studies rats were fed 84 nmol of 1-^{14}C-labeled oleic acid and killed 2 h later. The obvious and surprising feature is the consistency between regions despite large differences in LPL activities (also expressed per gram of tissue). These levels of uptake are substantially less than levels at the same 2 h time point in adipose tissue (0.097 nmol/g), and muscle (0.038 nmol/g) seen in studies where rats were fed 42 nmol of labeled oleic acid.

DISCUSSION

These studies indicate that within the CNS LPL is present in many brain regions; however, the most abundant source is the caudal spinal cord. This enzyme activity was demonstrated to be authentic LPL activity. The high levels of LPL activity in the caudal spinal cord are associated with high levels of LPL mRNA. Additionally, within the spinal cord LPL activity increases with feeding as it does within adipose tissue. Most of the LPL within the brain is found in blood vessels, although the cell bodies within the hippocampus and Purkinje cells in the cerebellum also contain the enzyme. In the caudal spinal cord, LPL is located around nerve fibers, in blood vessels, and to a lesser degree within axons themselves. LPL mRNA is found in a number of sites including the hippocampus, Purkinje cells, cells deep in the cerebral cortex, and in the white matter of the spinal cord where it could be made by either glial cells or neurons. Finally, it appears that there is uptake of dietary fatty acids within the CNS which is surprisingly consistent in all CNS regions examined. These studies raise questions as to the mechanisms that control the localization of LPL within the CNS and its function there.

Within various regions of the neuroaxis it appears that differences in LPL mRNA levels parallel changes in enzyme activity. This could be taken as evidence that within the neuroaxis transcriptional activation of the LPL gene or differences in message stability play an important role in determining regional LPL activity. This finding contrasts with previous studies on the regulation of adipose tissue LPL in response to feeding and fasting (16), obesity, and reduced-obesity (11, 17, 18). In these prior studies, while some modulation of LPL activity is associated with changes in steady state LPL mRNA levels, more often the important determinants of LPL activity are post-translational. Additionally, in the current studies, the uptake of dietary fat by various regions was found to be remarkably consistent from region to region. This could be taken as evidence that the delivery of TG fatty acids to regions of the CNS is important in determining the expression of the LPL gene at that site.

Despite the parallel between LPL mRNA and activity in the CNS, in several regions immunohistochemical studies showed that LPL protein was not located near cells that contained abundant LPL mRNA. There was very strong and consistent staining of basal vessels near the hypothalamus; however, very little staining of the adjacent hypothalamic parenchyma was seen in the in situ hybridization studies. In the upper spinal cord the immunostaining was strongest in the grey matter while hybridization of the riboprobe was strongest in the white matter. This raises the possibility that some of the LPL protein seen at these sites was actually synthesized elsewhere. Wallinder et al. (19) have shown that iodinated LPL injected intravascularly is rapidly cleared from the circulation, predominantly by the liver, but significant amounts are cleared by other tissues. The proteoglycans within the CNS have a high affinity for LPL (20). Saxena, Witte, and Goldberg (21) demonstrated that the products of TG hydrolysis displace LPL from its endothelial binding sites. It is possible then that LPL released into the circulation from peripheral tissues during the process of TG hydrolysis could travel to and bind to endothelial sites within the CNS. Such a process has been shown for hepatic triglyceride lipase and the adrenal gland and ovaries (22, 23).

What is the function of LPL within the CNS? The localization of LPL at the capillary endothelium of the CNS as well as the results of studies where [^{14}C]oleic acid was physiologically incorporated into chylomicron TG suggest that LPL within the CNS functions as it does in other sites, to deliver TGFA to cells in the immediate vicinity of the hydrolysis reaction. This delivery of dietary fatty acids to the CNS may be important in the development and maintenance of normal neuronal function. In the retina the presence of docosahexaenoic acid (22:6) derived from the diet is critically important for normal retinal function (24). Uauy-Dagach et al. (25) have recently shown that dietary essential fatty acids are critically important for the normal function of the human infant retina, the development of normal synaptic transmission from the retina to the visual cortex, and in complex visual

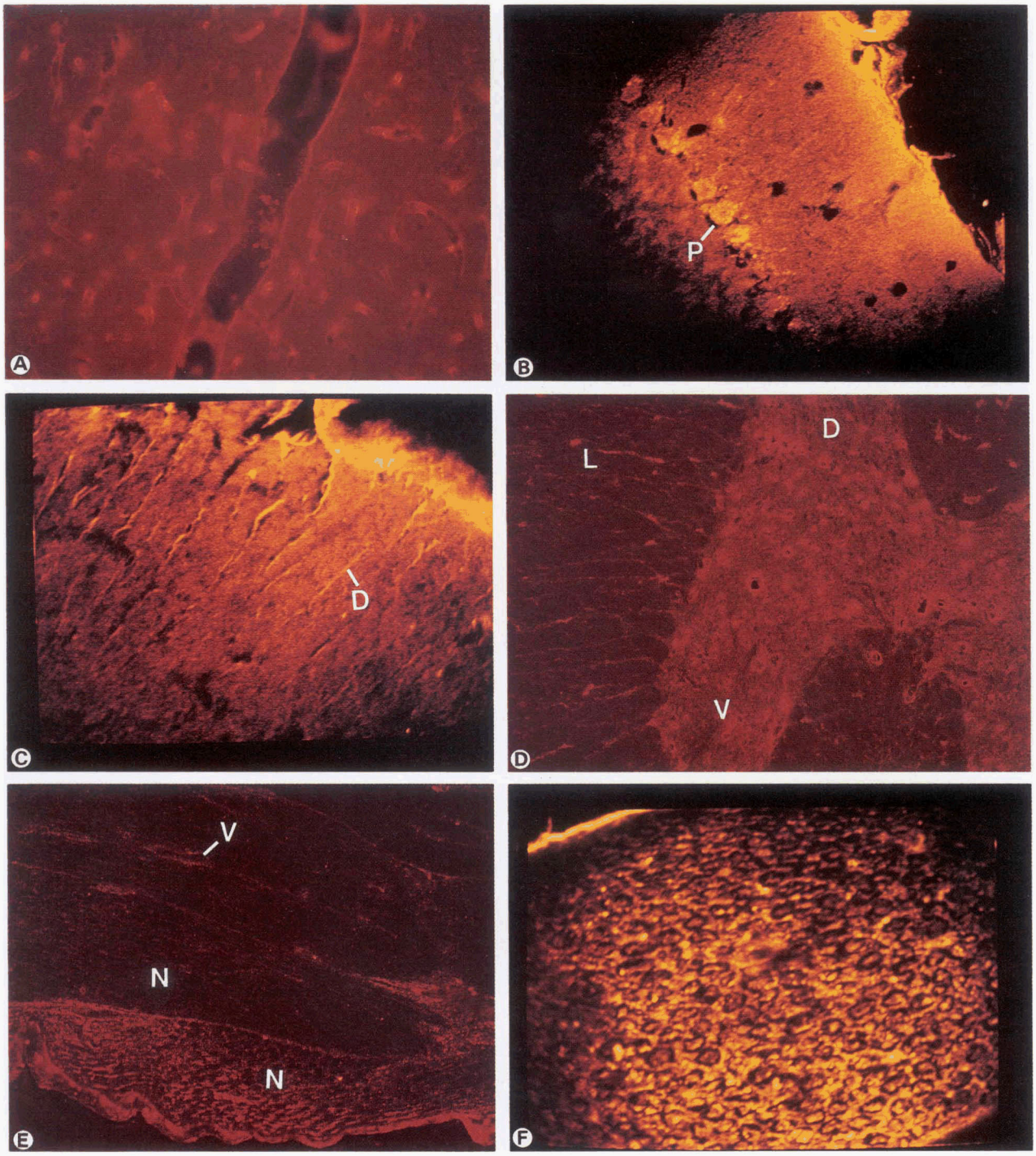

Fig. 3. Immunohistochemistry for LPL within rat brain and spinal cord. Frozen sections (16 μm) were incubated with a polyclonal anti-LPL primary antibody, followed by a rhodamine-conjugated secondary F(ab')2. Immunostaining of blood vessels of all sizes was seen in many brain regions. Fig. 3A demonstrates vascular staining in a region of the deep cortex. In the cerebellar cortex, strong staining of Purkinje cell bodies (Fig. 3B, P) and their associated dendritic trees (Fig. 3C, D) were seen. In the spinal cord (Fig. 3D) staining was mostly found in the dorsal (D) and ventral (V) horns of the gray matter. Relatively little staining was seen in the lateral funiculus (L). In the cauda equina (Fig. 3E) staining was seen in all nerve bundles (N); however, immunostaining was not evenly distributed, with some nerve bundles staining more than others. Central vasa nervorum stained strongly (V). Distal nerve bundles (Fig. 3F) showed staining around and within individual axons with a clear space interposed between. Photomicrographs were taken with either an Olympus BH-2 fluorescence microscope (Fig. 3A, D, E) or a Nikon microscope equipped with Bio-Rad confocal optics (Fig. 3B, C, F).

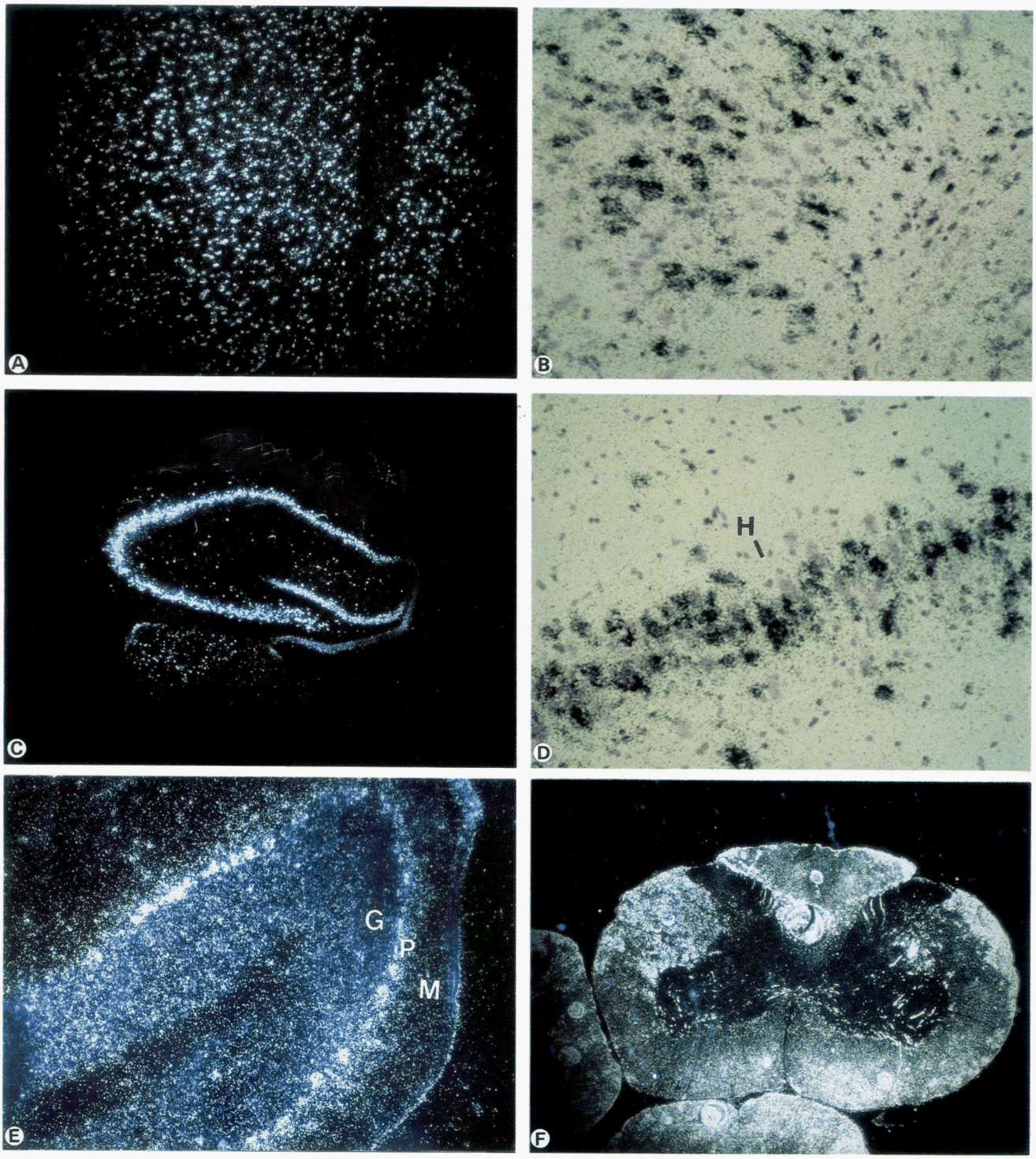

Fig. 4. Localization of LPL mRNA within regions of the rat brain and spinal cord by in situ hybridization. Frozen sections were hybridized with an anti-sense ^{35}S-labeled LPL cRNA. Specific labeling of a subset of neurons deep in the cerebral cortex is shown (Fig. 4A, darkfield illumination; Fig. 4B, brightfield illumination). Hybridization was also seen in most cells of the hippocampus Fig. 4C and D, H). In the cerebellar cortex (Fig. 4E) strong hybridization was seen over Purkinje cells (P) interposed between the molecular (M) and granular (G) layers. In the spinal cord, the strongest signal came from the white matter (Fig. 4F).

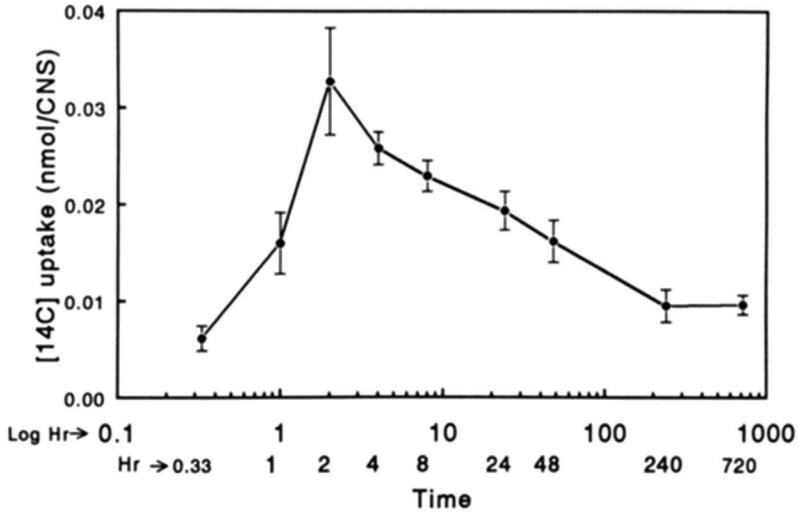

Fig. 5. Uptake of ^{14}C-labeled dietary oleate by the CNS over 30 days. Fasted Sprague-Dawley rats were fed 42 nmol of [1-^{14}C]oleic acid and killed 1, 2, 4, 8 h or 1, 2, 10, or 30 days later (n = 4 at each time point). The neuroaxis (brain and spinal cord) was removed and its ^{14}C content was determined. The data are presented as nmol ^{14}C-labeled fatty acid found in the entire CNS (mean ± SEM) as a function of the log of time in hours. The specific time points at which data were collected are highlighted under the logarithmic time scale.

behavior. The mechanisms that are responsible for the delivery of these dietary fats to the CNS have yet to be defined, but an LPL-mediated pathway may play an important role.

The delivery of dietary fat to the CNS may also play an important role in CNS function in adult rats as evidenced by the studies of Greenwood (26). In a variety of learning tasks she found that varying the fat content of the diet affected rats' ability to learn (27) as well as influencing dietary macronutrient selection (28). TGFA taken up by the CNS might be incorporated into phospholipid or might be oxidized. The time course of ^{14}C content found in the CNS following ingestion of labeled oleate suggests that about 30% of the dietary fat taken up is either oxidized or redistributed to other tissues. The remainder appears to persist, suggesting more stable incorporation into CNS lipids.

By delivering triglyceride fatty acids to neurons, LPL within the CNS might be involved in 'chemosensing' circulating levels of TGFA. Campfield et al. (29) have recently shown in free-feeding rats that meal initiation is preceded by a fall in plasma TG. These data suggest that there is accurate minute to minute 'sensing' of plasma TG levels, a role that could be performed by "TG-sensitive neurons" that might alter neuronal activity in response to the uptake of TGFA. A model for this kind of metabolic chemosensing neuron can be found in the glucose-sensitive and fat-sensitive neurons described by Oomura and colleagues (30–32).

What is the role of LPL found in the hippocampus and the Purkinje cells of the cerebellum where most of the protein appears by immunohistochemistry to be inside neurons and not bound to vascular endothelium? The strong staining of these cell bodies raises the possibility that LPL has an intracellular function in these cells. Oscai, Caruso, and Wergeles (33) have suggested that in skeletal muscle LPL may catalyze the hydrolysis of intracellular triglyceride stores; however, other authors find this unlikely as

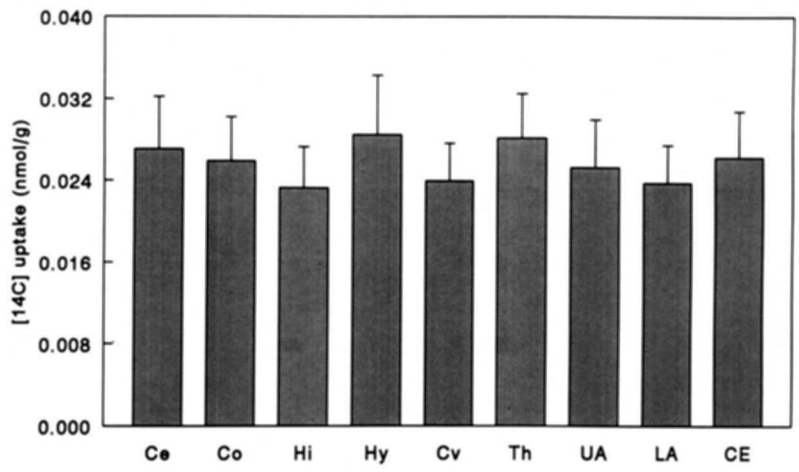

Fig. 6. Uptake of ^{14}C-labeled dietary oleate by different regions of the CNA at 2 h. For these studies, fasted rats were fed 84 nmol of 1-^{14}C-labeled oleate and killed 2 h later. Brain and spinal cord regions were isolated and their ^{14}C content was determined (n = 5). The regions examined include: cerebellum (Ce), cerebral cortex (Co), hippocampus (Hi), hypothalamus (Hy), and five regions of the spinal cord: cervical (Cv), thoracic (Th), upper abdominal (UA), lower abdominal (LA), and caudal spinal cord and associated nerve rootlets (CE). The data are presented as nmol of ^{14}C per gram of tissue (mean ± SEM). There are no significant differences between groups.

apoC-II is not present within the cell and is thought to be a necessary cofactor for TG hydrolysis catalyzed by LPL (34). Alternatively, LPL has been shown to have phospholipase activity in vitro (35, 36) and intracellular LPL may function in this manner at these sites.

Why are the levels of LPL highest in the caudal spinal cord and associated nerve rootlets? The data from the [^{14}C]oleate feeding studies suggest that higher levels of LPL are needed to deliver the same amount of TGFA to this area. This could be due to relatively less blood flow or lower fractional uptake of the products of hydrolysis compared to other CNS regions. Another possibility is that actually the peripheral nervous system has a high requirement for TGFA and the findings relate to the sampling of the proximal portion of cells that extend into the periphery.

By examining the entire neuroaxis, LPL mRNA, protein, and enzyme activity has been localized to several brain regions but the highest abundance has been found in the spinal cord, in particular the caudal spinal cord. Although the function of LPL in these regions of the CNS remains speculative, it is clear from the ^{14}C feeding studies that carbons from dietary fat are taken up by the CNS in a time course very similar to the LPL-dependent uptake in other tissues. The possibility that LPL functioning within the CNS is providing information on the circulating level of TG is one that waits neurophysiologic confirmation, but if true would have relevance to feeding-related neural systems. If LPL is providing lipid for phospholipid synthesis or as oxidative substrate for a subset of neurons, this too could have important implications for neural function. Further studies in this area will need to focus on the specific biochemical fate of lipid taken up by various regions of the CNS, the LPL dependency of these processes, and the neural consequences of this uptake process.

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