Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 7604–7607, December 1984
Medical Sciences

Lipoprotein lipase is produced, regulated, and functional in rat brain

(fatty acids/triacylglycerol/hypothalamus/fasting)

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Communicated by David W. Talmage, August 6, 1984

ABSTRACT **Lipoprotein lipase (LP lipase, triacylglycero-protein acylhydrolase EC 3.1.1.34) activity was found in four dissimilar brain regions (hypothalamus, cortex, cerebellum, and midbrain) of adult male rats. Progressive accumulation of LP lipase activity in cultured fetal rat hypothalamic cells was also observed, indicating *de novo* synthesis of the lipase. The brain LP lipase activity was serum-dependent and was inhibited by 1 M NaCl and by protamine sulfate. Kinetic analysis revealed an apparent K_m of 0.79 mM very similar to that of rat adipose tissue LP lipase. That the lipase was functioning in the cultured brain cells was indicated by uptake and incorporation of radioactivity from tri([1-^{14}C]oleoyl)glycerol into cellular triacylglycerols, and into more polar lipids, such as phosphatidylcholine. Furthermore, brain LP lipase activity in adult rats was decreased in all four regions examined, most significantly in the hypothalamus, after 72 hr of food deprivation. Thus, authentic LP lipase is present in adult rat brain and can be synthesized by isolated brain cells *in vitro*. LP lipase also mediates the uptake of triacylglycerol fatty acids and their subsequent incorporation into cellular lipids of cultured brain cells. Decreased brain LP lipase activity after fasting suggests that this enzyme may be regulated by metabolic or nutritional factors. Because the largest changes in LP lipase activity in response to food deprivation occurred in the hypothalamus, the enzyme may have a role in hypothalamic control of food intake or in body-weight regulation.**

The brain is rich in lipids (>50% of dry weight) and has a high content of fatty acids, most of which are incorporated into phospholipids (40% of brain lipid) or glycolipids (35% of brain lipid) (1). Fatty acids contained in brain phospholipids, glycolipids, or other neutral lipids could be synthesized in the brain (2) or assimilated from the plasma compartment either from albumin-bound nonesterified fatty acids or from fatty acids liberated by triacylglycerol-rich lipoprotein (chylomicron or very low density lipoprotein) triacylglycerol hydrolysis. This hydrolysis would be dependent on lipoprotein lipase (LP lipase, triacylglycero-protein acylhydrolase, EC 3.1.1.34), an enzyme produced in a number of extracranial tissues, including muscle, adipose tissue, and lactating breast (3). Smaller amounts of LP lipase have been detected in rat brain (4). However, in studies of rabbit brain microvessels, the enzyme was examined only on the endothelial surface (5), the site of triacylglycerol-rich lipoprotein triacylglycerol hydrolysis in other tissues (3).

Brain LP lipase could not only be an important source of fatty acids for brain cell lipid but could also serve as a metabolic regulator. For instance, increases in LP lipase-mediated fatty acid delivery to the hypothalamus could be a mechanism by which alterations in food intake are regulated. We provide evidence that LP lipase is present in several brain regions, is synthesized by and functional in brain cells *in vitro*, and is subject to regulation *in vivo* by fasting. It is the latter observation that strongly suggests a regulatory role of the enzyme in the brain.

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MATERIALS AND METHODS

LP Lipase Assay. LP lipase activity from brain fragments or from cell cultures was determined using an emulsified [^{14}C]triolein [tri([1-^{14}C]oleoyl)glycerol] substrate (0.7 mM, final concentration) and pooled human serum LP lipase activator as described (6). LP lipase released from cultured hypothalamic cells or from 40–45 mg of brain pieces by sodium heparin (Fisher; 13.3 μg/ml, 45 min, 37°C) was quantified as the amount of [^{14}C]oleic acid generated during the initial 90 min of the LP lipase assay. Assay sensitivity for heparin-released LP lipase was 0.01 nmol·min^{-1} per 10^6 cells for cultured cells and 0.15 nmol·min^{-1}·g^{-1} for brain fragments. Sensitivity of detection in culture medium was 0.03 nmol·min^{-1} per 10^6 cells.

Hypothalamic Cell Cultures. Hypothalamic cell cultures were prepared from the ventral diencephalons of 16-day-old rat embryos, using techniques previously reported for culturing rat telencephalic cells (7). The hypothalamic cells were prepared by enzymatic (neutral protease/papain/deoxyribonuclease) dispersal of a fragment of embryonic brain obtained as follows: fetal brains were placed on their dorsal surfaces and 1-mm-deep cuts were made along the lateral hypothalamic sulci, anterior to the optic chiasm, and caudal to the mammillary bodies. Choroid lining the third ventricle and surface meninges were removed prior to enzymatic dispersal. Polystyrene flasks (Corning, 25 cm^2) were each seeded with 5×10^6 cells on day 0. Cultures were maintained in minimal essential medium (Eagle) with Earle's salts plus 10% fetal calf serum at 37°C in a 5% CO_2 atmosphere. After 96 hr, the fetal calf serum was replaced with heat-inactivated horse serum. (All media and sera were obtained from GIBCO.) These cultures are morphologically heterogeneous, containing neuronal, glial, meningeal, choroidal, and, possibly, vascular cells. The non-neuronal cells proliferate and cover the surface of the flask by 96 hr. Neuronal morphology is mature and stable between days 8 and 20 *in vitro*. Cells from one 8-day-old culture contain ≈1.2 mg of protein (Bio-Rad protein assay). Cell viability, determined by trypan blue exclusion at day 8, is 95–97%. The hypothalamic origin of the cells was examined by assaying for the hypothalamic releasing factor, somatostatin. Immunoreactive somatostatin was present in cell extracts at days 7 (176 ± 9.0 pg per flask), 14 (210 ± 6.7 pg per flask) and 21 (260 ± 7.7 pg per flask).

Triacylglycerol Fatty Acid Incorporation into Cell Lipids. Two to 3 weeks after plating, cultured brain cells were variably exposed to emulsified [^{14}C]triolein substrate. Cells were then washed three times, scraped into phosphate-buffered saline, and separated into lipid and aqueous phases by Folch extraction (8). After precipitation of polar lipids with

Abbreviation: LP lipase, lipoprotein lipase.

0.1 g of silicic acid (9), both nonpolar and resuspended polar lipids were fractionated by thin-layer chromatography and detected by autoradiography.

Whole Brain Fragments. The presence of LP lipase in adult brain was determined in adult Sprague–Dawley rats (219 ± 16 g, range 147–310 g) who either had unlimited access to standard rat lab chow and water (fed) or were deprived of rat chow but not water for 72 hr (fasted). Rats were anesthetized and then perfused with ice-cold phosphate-buffered saline via the left cardiac ventricle to remove blood from the brain. Brains were rapidly removed and dissected at 4°C into the following major regions: frontal cortex, hypothalamus, cerebellum, and midbrain. Regional dissections using standard surface landmarks were performed using the method of McEwen and Pfaff (10). Samples (40–45 mg) from each region were kept at 4°C, weighed, minced, and immediately incubated in Krebs–Ringer solution containing heparin.

The Wilcoxin matched-pairs signed-ranks test and the Mann–Whitney *U* test were used for statistical analyses.

RESULTS

Hypothalamic Cell Cultures. LP lipase in medium that had been added to cultures 24 hr before measurement rose from undetectable amounts on day 1 to 0.40 ± 0.08 nmol·min^{-1} per 10^6 cells (±SEM, n = 4) by the third week *in vitro* (Fig. 1). No cultures older than this were examined.

LP lipase released from cell monolayers into Krebs–Ringer solution containing heparin (45 min incubation) likewise rose from undetectable levels on day 0 to 0.39 ± 0.07 nmol·min^{-1} per 10^6 cells (n = 6) by the third week *in vitro*. The dependence of LP lipase activity on protein synthesis was shown in 2-week-old cultures by 73 ± 7% (n = 2) and 74 ± 1% (n = 2) decreases in heparin-released LP lipase 2 and 4 hr, respectively, after the addition of cycloheximide (1 μg/ml) to the cultures. To investigate the dependency on serum activators, we omitted serum from the assay: heparin-released LP lipase activity decreased from 0.21 ± 0.05 to 0.05 ± 0.02 (n = 11). As shown for LP lipase in other systems (3), no LP lipase activity was detected when 1 M NaCl or protamine sulfate (3 mg/ml) was added to the assay.

Triacylglycerol Fatty Acid Incorporation into Cell Lipids. Exposure of cultured brain cells to emulsified [^{14}C]triolein substrate resulted in time-dependent uptake and incorporation of label into cellular lipids. Incorporation into total cellular lipids was 0.16 ± 0.06% (n = 6) at 0.5 hr, 0.36 ± 0.07% (n = 7) at 2 hr, and 0.99 ± 0.32% (n = 4) at 24 hr.

To determine the fate of incorporated label, both the polar and the nonpolar lipid fractions were subjected to thin-layer chromatography and autoradiography. A solvent system of petroleum ether, diethyl ether, and acetic acid (160:40:2, vol/vol) was optimal for separation of nonpolar lipids. At 0.5 hr, incorporation into nonpolar lipids had occurred (>200 cpm above background) but was below the level of detection by autoradiography. At 24 hr, incorporation into cellular triacylglycerol was seen (Fig. 2). The two radioactive bands in the region of fatty acid migration appeared to be oleic acid and a more polar fatty acid, resulting from either desaturation or fatty acid chain-length shortening. Both of these processes take place in brain (2).

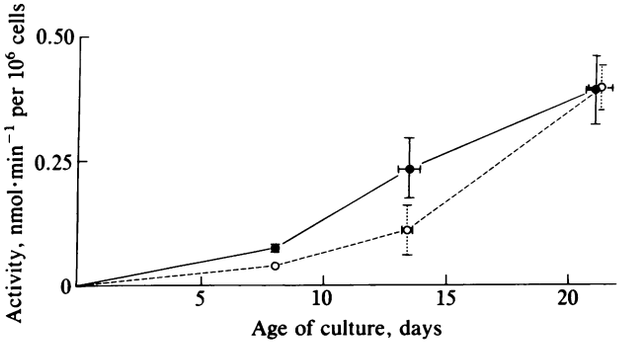

FIG. 1. LP lipase activity in cultures of ventral diencephalic cells from 16-day-old rat embryos, measured as enzyme activity in culture medium (○) or that released from cell monolayers with sodium heparin (13.3 μg/ml) (●). Data are expressed as the mean ± SEM (n = 4).

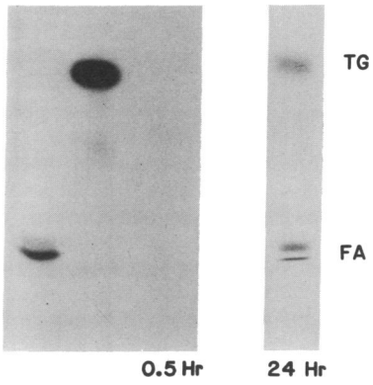

FIG. 2. Incorporation of [^{14}C]triolein radioactivity into brain cell nonpolar lipids. Two to 3 weeks after plating, cultured brain cells were exposed to emulsified [^{14}C]triolein substrate for 0.5 or 24 hr. Shown are autoradiographic patterns obtained after thin-layer chromatography of (from left) [^{14}C]oleic acid, the emulsified [^{14}C]triolein substrate, and nonpolar lipids from cells labeled for 0.5 or 24 hr. Migration positions of triacylglycerols (TG) and fatty acids (FA) are indicated on the right. Solvent system: petroleum ether/diethyl ether/acetic acid (160:40:2, vol/vol).

Polar lipids were optimally separated by thin-layer chromatography in chloroform/methanol/water (140:50:8, vol/vol) (Fig. 3). The radioactivity in the triacylglycerol area and in a fatty acid area (Fig. 3, 24-hr lane) is due to incomplete removal of nonpolar lipids by the silicic acid precipitation step prior to chromatography. At 0.5 hr, radioactivity was present in silicic acid-precipitated lipids but was not detectable by autoradiography. By 24 hr, incorporation into phosphatidylcholine (PC), seen as a faint band, and sphingomyelin (SM) was observed.

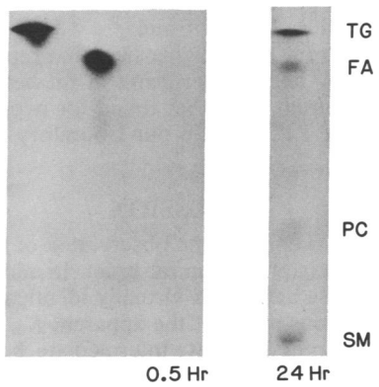

FIG. 3. Incorporation of [^{14}C]triolein radioactivity into brain cell polar lipids. Cultures were exposed to emulsified [^{14}C]triolein substrate as described in the legend to Fig. 2. Shown are autoradiograms obtained after thin-layer chromatography of (from left) [^{14}C]oleic acid, [^{14}C]triolein, and polar lipids from cells labeled for 0.5 or 24 hr. At 24 hr, ^{14}C incorporation from emulsified [^{14}C]triolein was seen in both the more polar lipids, including phosphatidylcholine (PC) and sphingomyelin (SM), and the less polar triacylglycerols (TG) and fatty acids (FA). Solvent system: chloroform/methanol/water (140:50:8, vol/vol).

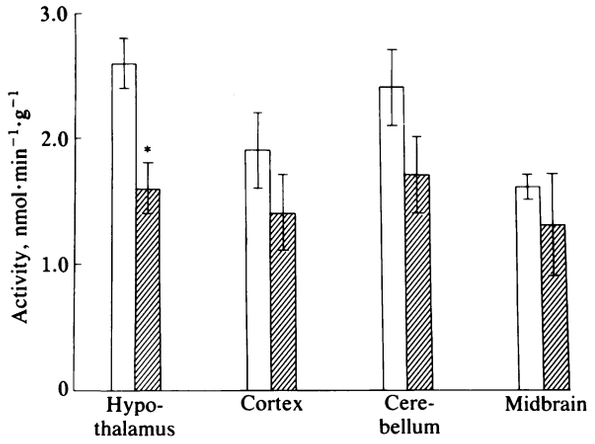

FIG. 4. LP lipase activity in brain tissue from adult rats. Enzyme activities released from brain pieces by sodium heparin (13.3 μg/ml) are shown for four brain regions. Open bars indicate LP lipase activity in rats given free access to food, while hatched bars indicate levels in rats fasted for 72 hr prior to sacrifice. Experimental data from paired experiments are expressed as mean ± SEM for hypothalamus (n = 7), cortex (n = 7), cerebellum (n = 7), and midbrain (n = 2). The asterisk indicates that the difference between the LP lipase activities in the hypothalamus of fed and fasted rats is significant ($P < 0.05$).

Whole Brain Fragments. LP lipase activity (expressed as nmol·min^{-1}·g^{-1}) was similar in all brain regions in fed animals: 1.8 ± 0.1 in midbrain (n = 4), 2.2 ± 0.2 in cortex (n = 12), 2.5 ± 0.2 in cerebellum (n = 12), and 2.3 ± 0.3 in hypothalamus (n = 16). In paired experiments (Fig. 4), LP lipase activity was lower in each brain area in fasted rats but significantly lower in the hypothalamus (fed, 2.6 ± 0.2; fasted, 1.8 ± 0.2; n = 7, $P < 0.05$). As with the LP lipase activity from hypothalamic cultures, the activity released from the adult brain pieces by heparin was inhibited by 1 M NaCl (to 5% of control) and by protamine sulfate at 3 mg/ml (to 15% of control) and decreased to 20% of control when serum was omitted from the assay.

Kinetic analysis of the enzyme activity released from hypothalamic fragments by heparin was carried out by incubation of LP lipase with various amounts (0.05–3.5 mM) of [^{14}C]triolein substrate. By Lineweaver–Burke transformation of saturation plots, an apparent K_m of 0.79 ± 0.12 mM and a V_{max} of 6.4 ± 1.9 nmol·min^{-1}·g^{-1} (n = 6) were obtained. This apparent K_m is higher than that reported for LP lipase released by heparin perfusion of rat heart (0.4 mM) (11), but not different from that found for heparin-released rat adipose tissue LP lipase in our laboratory (0.93 ± 0.30 mM).

DISCUSSION

These experiments confirm the observation of Chajek *et al.* (4) that LP lipase is present in rat brain. In addition, we report that LP lipase activity is virtually identical in four dissimilar brain regions and that the apparent K_m is within the range found for other tissues, intermediate between heart (11) and adipose tissue.

The ability of cultured brain cells to incorporate [^{14}C]triolein fatty acids into cell lipids strongly suggests a LP lipase-dependent process. LP lipase-mediated delivery of fatty acids for brain lipid synthesis could contribute to the overall need of brain for fatty acids. Dhopeshwarkar and Mead (12) have shown that the uptake of [^{14}C]palmitate into brain lipid is at least partially dependent on the delivery of the fatty acid in the form of triacylglycerol-rich lipoprotein.

LP lipase activity was also regulated *in vivo* by fasting. Although LP lipase activity decreased in all brain regions, the largest decrease occurred in hypothalamic LP lipase. LP lipase-dependent fatty acid uptake into brain cells in the hypothalamus is particularly noteworthy because food intake and body weight are to a large extent regulated in this brain region (13, 14). Lesions in the ventromedial nucleus of the hypothalamus in rats result in increased food intake and weight gain (15); similar lesions in the lateral hypothalamus variably cause decreases in food intake and weight loss (15, 16). After such lesions occur, adaptation and regulation of food intake and body weight at a new level then occur. Based on these observations, an integrated hypothesis might relate LP lipase-dependent fatty acid uptake in the ventromedial nucleus to suppression of food intake by as yet unidentified intra- and intercellular events. Alternatively, decreases in LP lipase-mediated fatty acid uptake during fasting could stimulate increases in food intake. Because the entire hypothalamus was used for our studies, such hypothesis would suggest a predominant regulatory role of the ventromedial nucleus over the lateral hypothalamus in such a process.

The observation that heparin-released LP lipase increases with time in isolated hypothalamic cells *in vitro* and that the activity of the enzyme is inhibited by cycloheximide verify that cells in this brain region have the capacity to synthesize the lipase activity *de novo*. The loss of activity in the absence of serum, as well as the inhibition by NaCl and protamine sulfate, confirm that this activity is authentic LP lipase (3).

Although human neuroblastoma cell line NBP_2 (17) contained only small amounts of LP lipase activity (0.02 ± 0.02 nmol·min^{-1} per 10^6 cells, n = 2), we have no direct evidence that glial cells are the source of the enzyme. An alternative source of LP lipase could be the microvascular endothelium. However, evidence for endothelial production of LP lipase in other tissues—e.g., aorta (18) and adipose tissue (19)—is lacking.

In conclusion, we have identified LP lipase activity in several brain regions, shown that isolated brain cells synthesize the enzyme, provided evidence for triacylglycerol fatty acid incorporation into brain cell lipids, and shown *in vivo* regulation of LP lipase in response to the metabolic challenge of fasting. Thus, LP lipase may not only be important in the regulation of brain lipid metabolism but could serve as a regulator or mediator of certain hypothalamic functions, including body-weight regulation and appetite control.

We thank Roxanne Landon and Judith Prasad for technical assistance and Wendy Hall for secretarial skills. Supported by National Institutes of Health Grants AM 32226, AM 30451, and BRSG-05357.

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