Expression and Regulation of the Lipoprotein Lipase Gene in Human Adrenal Cortex*

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Lipoprotein lipase (LPL), an enzyme which hydrolyzes triglycerides and participates in the catabolism of remnant lipoproteins, plays a crucial role in energy and lipid metabolism. The goal of this study was to analyze the expression and regulation of the LPL gene in human adrenals. Reverse transcriptase-polymerase chain reaction amplification and sequence analysis demonstrated the presence of LPL mRNA in fetal and adult human adrenal cortex. Furthermore, the human adrenocortical carcinoma cell line, NCI-H295, expresses LPL mRNA and protein, which is localized to the outer cellular membrane as demonstrated by immunofluorescence confocal microscopy and can be released in the medium by heparin stimulation. To assess whether the LPL gene is regulated by agents regulating adrenal steroidogenesis, NCI-H295 cells were treated with activators of second messenger systems. Whereas the calcium-ionophore A23187 did not affect LPL gene expression, treatment with phorbol 12-myristate 13-acetate decreased LPL mRNA levels in a time- and dose-dependent manner. This decrease after phorbol 12-myristate 13-acetate was associated with diminished heparin-releasable LPL mass and activity in the culture medium. Addition of the cAMP analog 8-Br-cAMP to NCI-H295 cells resulted in a rapid, but transient dose-dependent induction of LPL mRNA. Treatment with the protein synthesis inhibitor cycloheximide gradually induced, whereas simultaneous addition of cAMP and cycloheximide superinduced LPL mRNA levels. Nuclear run-on analysis indicated that the effects of cAMP and cycloheximide occurred at the transcriptional and post-transcriptional level, respectively. Transient co-transfection assays demonstrated that the first 230 base pairs of the proximal LPL promoter contain a cAMP-responsive element activated by protein kinase A and transcription factors belonging to the CREB/CREM family. These data indicate that LPL is expressed in human adrenal cortex and regulated in NCI-H295 adrenocortical carcinoma cells by activators of the protein kinase A and protein kinase C second messenger pathways in a manner comparable to P450ccc, which catalyzes the first step in adrenal steroidogenesis. These observations suggest a role for LPL in adrenal energy and/or lipid metabolism and possibly in steroidogenesis.

The adrenal cortex is the major site of the biosynthesis of steroid hormones, such as glucocorticoids and mineralocorticoids (for review, see Ref. 1). Adrenal steroidogenesis is controlled by the action of specific peptide hormones (2). In the adrenal cortex, glucocorticoid synthesis is stimulated by the anterior pituitary-derived adrenocorticotropic hormone (ACTH),* which, after binding to a specific cell surface receptor, activates adenylate cyclase, resulting in elevated intracellular cAMP concentrations and activation of the protein kinase A (PKA) signal transduction pathway (2). Mineralocorticoid synthesis, on the other hand, is induced by angiotensin-II, which activates the protein kinase C (PKC) pathway of intracellular second messengers. The first step in the enzymatic conversion of cholesterol into steroid hormones is catalyzed by the mitochondrial cytochrome P450 side chain cleavage enzyme (P450ccc), which catalyzes the conversion of cholesterol into pregnenolone. Long term regulation of steroidogenesis by these peptide hormones occurs via changes in P450ccc gene expression and transcription (3-5). Human adrenals derive the cholesterol necessary for steroid hormone synthesis mainly by receptor-mediated endocytosis of low density lipoproteins (LDL) and, to a lesser extent, by de novo synthesis, the rate-limiting step of which is catalyzed by the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (6-8). Although patients homozygous for familial hypercholesterolemia have a mild impairment in cortisol secretion during maximal ACTH stimulation (9), the absence of adrenal dysfunction in patients with defects in the LDL-receptor pathway treated with hydroxy 3-methylglutaryl-coA reductase inhibitors, such as mevinolin, suggested the existence of alternative pathways of cholesterol delivery to the adrenal for steroid synthesis (10).

Lipoprotein lipase (LPL) occupies a pivotal position in both

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The abbreviations used are: ACTH, adrenocorticotropic hormone; apo, apolipoprotein; CAT, chloramphenicol acetyltransferase; CHX, cycloheximide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LPL, lipoprotein lipase; PKA, protein kinase A; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; P450ccc, cytochrome P450 side chain cleavage enzyme; PIPES, 1,4-piperazinediethanesulfonic acid; VLDL, very low density lipoprotein; PCR, polymerase chain reaction; bp, base pair(s); RT, reverse transcription; CREB, CAMP response element-binding protein(s); CREM, CAMP response element modulator.
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lipoprotein and energy metabolism. Located as a homodimer on the capillary endothelium of tissues, LPL hydrolyzes triglycerides in chylomicrons and very low density lipoprotein (VLDL) particles using apoc-II as a co-factor (11). The released free fatty acids are taken up by the underlying tissue for oxidation to generate ATP (muscle), for storage (adipose tissue), or for secretion in milk (mammary gland). In addition to its lipolytic activity on triglyceride-rich lipoprotein particles, LPL functions as a ligand for the VLDL receptor (12), the LDL receptor-related protein (13), and the LDL receptor (14–16), implicating a role for LPL in the uptake of cholesterol-rich remnant particles (for review, see Ref. 17). The cDNA (18), genomic structure (19, 20), and chromosomal location (chromosome 8 (21)) of the human LPL gene have been determined. LPL is expressed in a number of differentiated tissues, such as adipocytes, macrophages, lactating mammary gland, and muscle (for review, see Ref. 11). In contrast, in liver LPL is only expressed in fetal and neonatal liver and becomes extinguished 3 weeks after birth (22, 23).

In view of the pivotal role of LPL both in energy and lipoprotein metabolism, the main objective of the current investigation was to analyze whether LPL is expressed in the human adrenal cortex, a major cholesterol metabolizing tissue. Our results demonstrate the presence of LPL mRNA both in fetal and adult adrenal cortex. Furthermore, LPL is expressed in the recently characterized human adrenocortical carcinoma cell line, NCI-H295 (24, 25), where it is located on the plasma membrane and can be released by heparin treatment. LPL expression in NCI-H295 cells is regulated by cAMP and phorbol esters, activators can be released by heparin treatment. LPL expression in NCI-H295 cells is regulated by cAMP and phorbol esters, activators (for review, see Ref. 17). The cDNA (18), genomic structure (19, 20), and chromosomal location (chromosome 8 (21)) of the human LPL gene have been determined. LPL is expressed in a number of differentiated tissues, such as adipocytes, macrophages, lactating mammary gland, and muscle (for review, see Ref. 11). In contrast, in liver LPL is only expressed in fetal and neonatal liver and becomes extinguished 3 weeks after birth (22, 23).

EXPERIMENTAL PROCEDURES

Materials—Cycloheximide (CHX), transferrin, selenium, insulin and Heps were obtained from Boehringer Mannheim (Mannheim, Germany) and fatty acid-free bovine serum albumin, diethytritol, succrose, PIPES, glycerol trinitrate, 8-Br-cAMP, phorbol 12-myristate 13-acetate (PMA), and the calcium ionophore, A-23187, from Sigma. Superscript reverse transcriptase and cell culture media were from Life Technologies (Gaithersburg, MD). Glyceraldehyde tri-(9,10-in)-P-6-lactate was obtained from Amersham International and (α-32P)UTP, (α-32P)UTP, and P(32P)-cDNA labeling mix (containing 60 Ci/mmol) was obtained from ICN Nucleotides (Costa Mesa, CA). Poly(A)Quik push columns (Stratagene, La Jolla, CA). For analysis of LPL expression in adult and fetal adrenal and NCI-H295 cells, total RNA (50 ng) was reverse transcribed using random hexamer primers and Superscript reverse transcriptase. LPL mRNA was subsequently PCR amplified (35 cycles of 1 min at 94°C, 1 min at 57°C, 1 min at 72°C) using as primers the sense oligo GCA ATG GTC GTA CCA ATG GAG AGC AAA GCC CTTG (containing HindIII/KpnI cloning sites) and the antisense oligo TAC ATT CCT GTT ACC CTC CAG CCA TTG ATC (containing a Ncol cloning site), yielding a fragment of the expected size of 277 bp (18). This fragment spans exons 1 and 2 of the human LPL gene (19), which excludes potential contamination of the RNA samples with genomic DNA. RNA from HepG2 and differentiated THP-1 cells was negatively amplified as negative controls for LPL expression, respectively (26, 27). GAPDH-specific primers (sense primer: TGA TGA CAT CAA GAA GGT GGT CAA G; antisense primer: TCC TTG GAG GCC ATG TGG GCC AT) were used as control for the RT-PCR reaction (expected fragment size: 239 bp) (31). The resulting products were separated on a 2% agarose gel and subsequently stained with the pUC19 plasmid vector. Sequence analysis revealed complete identity to the previously reported human LPL cDNA sequence (18).

Northern and dot blot hybridizations of total cellular RNA and polysRNA were performed as described previously (23) using the human LPL cDNA clone, pLPL-26, as a probe (26). The human LPL cDNA probe was prepared by RT-PCR amplification based on the published sequence (32). A GAPDH probe was used as a control probe (31). All cDNA probes were labeled by random primed labeling (Boehringer Mannheim). Filters were hybridized to 1.5 × 106 cpm/ml of each probe as described (23). They were washed once in 0.5× SSC and 0.1% SDS for 10 min at room temperature and twice for 30 min at 65°C and subsequently exposed to x-ray film (X-Omat-AR, Kodak). Autoradiographs were analyzed by the method of Feinberg and Vogelstein (33). The different 5′-end-labeled RNA species were detected by phosphorimaging (Molecular Dynamics). For quantitative analysis the Signals were scanned and quantitated as described by Bio-Rad (34, 35). The amount of tissue DNA using psf55 plasmid vector. After 8 h of incubation cells were changed to fresh medium for another 16 h and cells were subsequently treated with 8-BR-cAMP (300 μmol/l) or solvent for a further 6 h. CAT and luciferase activities were determined on cell extracts as described (35, 36).

LPL Mass and Activity Assays—LPL mass was determined by a solid phase sandwich enzyme-linked immunosorbent assay using a chicken polyclonal antibody against LPL for coating and the 2D monoclonal antibody for detection as described (37). LPL activity was determined by the method of Ramirez et al. (38) with minor modifications. The reaction mixture contained 0.25 μmol of 8-Br-cAMP (10 μmol/l), 50 μmol/100 μmol/l of bovine serum albumin, 3% rat serum (preheated for 30 min at 37°C), 25 μmol PIPES (pH 7.5), and 0.02 M of sample in a final volume of 0.2 ml. After incubation for 30 min at 37°C, the reaction was stopped and [3H]oleate was separated from the substrate and quantified as described previously (39). The amount of enzyme catalyzing the release of 1 μmol/min of deacyt is defined as 1 unit.

Western Blotting—To obtain NCI-H295 cell extracts, cells were lysed in a buffer containing 1% (w/v) Triton X-100, 5 μmol/l leupeptin, 2 μmol/100 μmol/l leupeptin (Sigma) in phosphate-buffered saline. The lysed cells were scraped, rapidly frozen in liquid nitrogen, and sonicated for 30 s at maximum power. Next, the extract was centrifuged 10 min at 13,000 rpm in an Heraeus Biofuge A centrifuge at 4°C. The supernatant (20 μl) was loaded onto a SDS-polyacrylamide gel. After electrophoresis, the gel was blotted to
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By Western blot analysis of total cell extracts using a specific monoclonal antibody against bovine LPL (5D2) a single band of an apparent molecular mass of 58–60 kDa was detected (Fig. 2A). The electrophoretic mobility of this band was identical to human LPL produced in COS1 cells transfected with the full-length human LPL cDNA (not shown) (18), thereby confirming that LPL is present in NCI-H295 cells. The absence of heparin LPL mass was undetectable in the absence of heparin (Fig. 3A). This increase was most pronounced when NCI-H295 cells were incubated in the presence of heparin sulfate-proteoglycans and protects it from degradation in the cell culture medium (11). In addition, heparin treatment resulted in the appearance of detectable LPL immunoreactivity in the cell culture medium (Fig. 3B). In the absence of heparin LPL mass was undetectable in the medium, which is most likely due to the lower sensitivity of the mass determination by enzyme-linked immunosorbent assay compared to the LPL activity assay (Fig. 3B).
LPL mRNA Is Regulated by Phorbol Ester, but Not by Calcium Ionophore—To examine LPL regulation by activators of the angiotensin-II signal transduction pathway, which modulates the expression of steroidogenic enzymes, NCI-H295 cells were treated with the phorbol ester PMA, an activator of the PKC second messenger pathway, and the calcium ionophore, A-23187, which mobilizes intracellular calcium. Similarly to P450scc (24), which catalyzes the first step in adrenal steroidogenesis, PMA treatment resulted in a time-dependent decrease in LPL mRNA levels, which was already apparent after 6 h and reached a nadir after 12 h of treatment (Fig. 4A). Furthermore, this decrease in LPL mRNA after PMA was dose-dependent (Fig. 4B). Under these conditions, GAPDH mRNA levels did not change significantly, thereby confirming our previous observations (24). The decrease in LPL mRNA levels after PMA was followed at 24 and 48 h after PMA by a decreased LPL activity (Fig. 5A) and secretion of LPL protein (Fig. 5B) in the cell culture medium. In contrast to treatment with PMA, addition of the calcium ionophore A-23187 did not change significantly LPL mRNA levels when compared to GAPDH mRNA (data not shown).

Regulation of LPL Gene Expression by cAMP—Next, it was examined whether LPL mRNA levels are regulated by cAMP, an activator of the PKA signal transduction pathway which mediates the adrenal response to ACTH. When NCI-H295 cells were incubated with the cAMP analog, 8-Br-cAMP, LPL mRNA levels increased rapidly (within 3 h) by 3-fold, but this induction was transient, already decreasing after 12 h and reaching baseline values within 24 h after addition of 8-Br-cAMP (Fig. 6A). When NCI-H295 cells were treated with the protein synthesis inhibitor, CHX, LPL mRNA levels increased more gradually reaching a maximum 3-fold induction after 12–24 h (Fig. 6A and B). Simultaneous treatment of NCI-H295 cells with 8-Br-cAMP and CHX, however, resulted in a tremendous superinduction of LPL mRNA levels leading to an approximately...
being maximal at a dose of 1000 μM (Fig. 7). As a similar dose-dependent effect of 8-Br-cAMP and CHX was observed on P450sc mRNA levels (Fig. 7).

CAMP Induces LPL Gene Expression at the Transcriptional Level, Whereas CHX Acts at the Post-transcriptional Level—To study the mechanism of induction of LPL gene expression by cAMP and/or CHX, nuclear run-on experiments were performed next. LPL gene transcription rates increased when NCI-H295 cells were treated for 2 h with 8-Br-cAMP (Fig. 8). CHX alone did not affect LPL transcription and simultaneous addition of CHX and 8-Br-cAMP resulted in an induction comparable to 8-Br-cAMP alone (Fig. 8). Similarly to LPL, P450sc mRNA gene transcription increased after 8-Br-cAMP independent whether CHX was present or not (Fig. 8). When NCI-H295 cells were treated for 6 h similar results were obtained: 8-Br-cAMP, but not CHX, induced LPL gene transcription and simultaneous addition of 8-Br-cAMP and CHX resulted in an induction comparable to 8-Br-cAMP alone (data not shown). These data indicate that cAMP treatment induces LPL expression at the transcriptional level, whereas LPL expression is regulated at a post-transcriptional level by protein synthesis inhibition.

PKA and the Transcription Factors CREB/CREM Activate LPL Gene Promoter Transcription in the Steroidogenic Cell Lines NCI-H295 and JEG-3—In order to test whether 5′-upstream regulatory sequences in the human LPL gene mediate the inductive effects of cAMP on LPL transcription, transient transfection experiments were performed next. When NCI-H295 cells were transfected with a luciferase expression vector driven by different 5′-deletions of human LPL gene promoter, all constructs were induced to a similar extent by cAMP, indicating the presence of a cAMP-response element (CRE) in the most proximal 230 bp (AN-LUC) of the human LPL gene promoter (not shown and Fig. 9A). To determine the role of transcription factors of the CREB/CREM family (42, 43), which are activated after phosphorylation by PKA (44, 45), the proximal 180-bp LPL gene promoter-containing AN-LUC vector was co-transfected with the activator CREM (46) and/or PKA expression vectors into NCI-H295 cells. Co-transfection of CREM alone activated LPL transcription 2-fold and addition of 8-Br-cAMP (300 μM) resulted in an enhanced 5-fold induction (Fig. 9A). When a wild-type PKA expression vector alone was co-transfected LPL promoter activity was induced approximately 3-fold. Co-transfection of PKA in the presence of CREM activated the LPL promoter to a similar extent compared to CREM and cAMP (Fig. 9A).

Although the human placenta expresses the LPL gene (49),...
JEG-3 cells do not contain endogenous LPL. Co-transfection of the –230-bp proximal LPL promoter-containing CAT vector with a wild-type PKA expression vector resulted in a small induction of CAT activity (Fig. 9B). This induction was not observed when a mutated PKA expression vector, which can no longer phosphorylate CREB/CREM, was co-transfected into these cells. Co-transfection with CREB resulted in a strong induction of the LPL promoter, which was further enhanced by wild-type PKA, but not by mutated PKA expression vector. These data indicate that transcription factors belonging to the CREB/CREM family can activate the LPL promoter in steroidogenic cells and may participate in the activation of LPL gene transcription by cAMP.

**DISCUSSION**

The results from this study show that the LPL gene is expressed in human fetal and adult adrenal cortex. Previous reports have indicated the presence of LPL mRNA in rat, guinea pig, and human total adrenal glands (18, 50–53). In rats, however, LPL expression was mainly confined to the adrenal medulla (52), whereas in guinea pig LPL immuno-activity was observed in both the cortex and the medulla and more specifically in a subpopulation of lipid-filled cells (53). Our results show that LPL mRNA and protein is present in the human adrenocortical carcinoma cell line, NCI-H295, and hence suggest that in man LPL is actually produced in the steroid-producing cells of the adrenal cortex.

In the human adrenocortical carcinoma cell line, NCI-H295, LPL expression is regulated by the activation of intracellular second messenger systems, which mediate the effects of specific tropic peptide hormones on adrenal steroidogenesis. PMA-induced activation of the PKC pathway, which negatively regulates steroidogenesis in adrenal cells (54), results in a time- and dose-dependent decrease in LPL mRNA levels which is accompanied by decreased secretion of LPL mass and activity in the cell culture medium. In contrast, mobilization of intracellular calcium ion, after treatment with the calcium ionophore A-23187, does not substantially alter LPL gene expression in these cells. This regulation of LPL expression in adrenal cells is comparable to the regulation of the first enzyme in adrenal steroidogenesis, P450sc, whose mRNA levels decrease after PMA, but remain unchanged after A-23187 (24). In contrast, in the human monocyte cell line THP-1 treatment with phorbol esters, which induces a more differentiated macrophage-like phenotype, is accompanied by the induction of LPL expression at the transcriptional level (26, 41). In addition, treatment of THP-1 cells with the calcium ionophore A-23187, induced LPL mRNA levels, possibly also via activation of PKC (41). In contrast, in the mouse hepatoma cell line, BWTG3, LPL expression appears to be refractory to regulation by stimulators of PKC (27). Therefore, LPL expression appears to be differentially regulated by activators of PKC in different tissues and cell lines.

Addition of cAMP to the culture medium results in a rapid induction of LPL mRNA in NCI-H295 cells. This induction of LPL expression in adrenal cells shows some similarity to the situation in rat mesenchymal heart cells as well as in the neonatal mouse hepatoma cell line BWTG3, where LPL activity
and mRNA levels are induced by activation of the cAMP signal transduction pathway (27, 55). In contrast, elevation of intracellular cAMP concentrations decreases LPL synthesis in rat and avian adipocytes (56, 57), but this decrease appears to occur at the (post)translational level (58). In the adrenal cortex, ACTH activates steroidogenesis resulting in an increased production of glucocorticoid hormones by the zona fasciculata and reticularis. By increasing adenylyl cyclase activity, ACTH increases intracellular cAMP concentrations resulting in the activation of PKA. In contrast to the induction by cAMP of most steroidoenzymes, such as P450sc, P450c17, and P450c21 (24), this induction is only transient and LPL mRNA levels return within 12–24 h after treatment to pretreatment baseline levels. Remarkably, addition of the protein synthesis inhibitor, CHX, induces LPL mRNA levels. In contrast to P450sc, whose induction is independent of ongoing protein synthesis (this study and Ref. 24), simultaneous addition of cAMP and CHX results in a marked superinduction of LPL gene expression. These results indicate that LPL expression is under control of a labile negative regulatory protein, which represses LPL expression. A similar situation is also observed in THP-1 cells, where CHX treatment induces LPL mRNA levels and simultaneous addition of PMA and CHX results in a superinduction of LPL mRNA, albeit less pronounced compared to NCI-H295 cells (41). The results from the nuclear run-on experiments with NCI-H295 cells indicate that CHX treatment does not induce LPL gene transcription, thereby indicating that this labile negative regulatory protein regulates LPL expression at a post-transcriptional level, most likely by affecting LPL RNA stability.

The nuclear run-on experiments indicate that cAMP rapidly (within 2 h) activates LPL (and P450sc) expression at the transcriptional level. Furthermore, transient transfection assays in NCI-H295 cells suggest that the cAMP-mediated activation of LPL transcription is mediated by a cis-acting element(s) located within the first 230 bp of the proximal human LPL promoter region. Co-transfection of wild-type, but not mutated, PKA expression vector by itself is sufficient to induce LPL gene transcription in NCI-H295 cells. Although the exact nature of the cAMP-response element and the transcription factors implicated in its activation remain to be determined, our co-transfection experiments suggest a functional implication of the cAMP-response element-binding proteins of the CREM/CREB family. In addition, transient transfection experiments performed in placenta-derived choriocarcinoma JEG-3 cells, another human steroidogenic cell line, indicate that these elements are functional and can be activated by PKA and CREB in steroidogenic cells derived from other tissues. Although JEG-3 cells do not express the endogenous LPL gene, the P450sc gene is expressed and activated by cAMP in these cells (47).

Although the transcriptional induction of steroidogenic enzymes after cAMP treatment appears to be mediated by cAMP-dependent protein kinases (2, 54, 59), the involvement of transcription factors belonging to the CREB/CREM family is presently unclear. Purified CREB protein has been demonstrated to bind to a near-classical CRE in the P450c11b and P450sc genes, but functional transactivation experiments have not yet been performed (60–62). Furthermore, CREB or related factors have been shown to regulate the P450c17 and P450c21 genes (63, 64). Since the transcription activation of steroidogenic enzymes has been shown to be relatively slow (several hours) in adult bovine adrenal cells (3), it has been suggested, however, that the CRE/CREB system might not be involved in the response to cAMP (65). In contrast to bovine adrenal cells, the induction of both LPL and P450sc gene transcription by cAMP in human NCI-H295 cells is rapid (within 2 h). Whether these differences are due to differences between species (human versus bovine) or cell model systems (primary adrenal cells versus adrenocortical carcinoma cells) is presently unclear, but the observations from this study suggest that the CRE/CREB system may be functionally involved in mediating the cAMP response of the LPL gene in human adrenal NCI-H295 cells. It is most likely that adrenal cortex basal and cAMP-activated gene expression requires cooperation between the classical CRE-binding proteins, CREB/CREM, different specific adrenal-enriched transcription factors, and ubiquitous transcription factors, such as Sp1, binding to cAMP-responsive elements and to adjacent sites (65). Specific adrenal-enriched transcription factors, such as NGFI-B/mur77, SF-1/Ad4BP, and DAX-1 (66–69), may enhance the cAMP responsiveness of various genes involved in steroidogenesis (62, 70–73). Whether these adrenal-enriched transcription factors participate in the basal and cAMP-mediated expression of the LPL gene awaits further studies.

This coordinate regulation of the LPL gene and the first enzyme of adrenal steroidogenesis, P450sc, suggests a functional role of LPL in adrenal steroidogenesis. Although the exact function of LPL in the adrenal gland cannot be deduced from this study, it is tempting to speculate about its potential role. Through its enzymatic activity, hydrolyzing triglycerides and releasing free fatty acids which can be taken up by the underlying tissue, LPL may help the cell to provide in its energy requirements. In this respect, it is interesting to note that LPL expression has been detected in (parts of) tissues displaying a high metabolic activity, such as muscle (74, 75). Alternatively, LPL may be involved in the uptake of lipoprotein cholesterol required for steroidogenesis by the adrenal gland, as has been suggested for rat adrenal cells (76). By locally increasing hydrolysis of VLDL triglycerides LPL may change the conformation of lipoprotein particles rendering them more accessible for binding to the receptors of the underlying tissue. Although the LDL receptor plays a major role in cholesterol delivery to the adrenal gland (6–8), the absence of adrenal dysfunction in patients with defects in the LDL receptor pathway upon treatment with hydroxy 3-methylglutaryl-CoA reductase inhibitors, has implicated the existence of alternative routes for cholesterol delivery to the adrenal (10). Separate from its enzymatic function, LPL has been shown to mediate binding of cholesterol-rich remnant lipoprotein particles to specific cell surface receptors, such as the LDL receptor-related protein, thereby mediating their uptake. In this respect, it is interesting to know that NCI-H295 cells express high levels of LDL receptor-related protein (3), which may, in part, explain the localization of LPL protein on the outer cell membrane. Finally, the fact that LPL expression is not confined to the adrenal, but is also observed in other steroidogenic tissues, such as the ovary, points to the requirement of this enzyme in other steroidogenic tissues (13).

In conclusion, these results demonstrate the presence of LPL mRNA in fetal and adult adrenal cortex and in the steroid-synthesizing adrenocortical carcinoma cells NCI-H295. In NCI-H295 cells LPL expression is regulated by cAMP and phorbol esters, activators of the PKA and PKC second messenger pathways, respectively, in a manner reminiscent of the regulation of the first enzyme in adrenal steroidogenesis, P450sc. These data therefore suggest a role for LPL in adrenal energy and lipoprotein metabolism.

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