Transcriptional control of the expression of lipoprotein lipase gene by growth hormone in preadipocyte Ob1771 cells

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Abstract A direct and modulating effect of growth hormone (GH) on the regulation of the lipoprotein lipase (LPL) gene has been shown in preadipocyte Ob1771 cells. Growth hormone acts as a modulator within the physiological range of concentrations and regulates the abundance of the two species of LPL mRNAs (3.3 and 3.7 kb) in a differentiation-dependent manner, the stimulation factor being between 4- and 7-fold. The regulation of LPL gene expression by GH is rapid (2 to 8 h) and similar for both mRNA species. It is reversible and takes place primarily at a transcriptional level. Parallel increases of LPL mRNAs, LPL protein, and LPL activity are observed. The expression of both cellular and secreted activities is stimulated by GH. The role of GH is mediated, at least in part, by means of activation of protein kinase C. In the presence of 4-β-phorbol-12-myristate 13-acetate (PMA), a parallel increase of LPL mRNA content and LPL activity is observed at half the values obtained upon stimulation by GH. The kinase inhibitor 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7) abolishes completely the PMA-induced accumulation but decreases only by half that induced by GH. Like H7, staurosporine, polymixin B, and sphingosine inhibit only by half the stimulatory effect of GH on the expression of the LPL gene. These results show for the first time a rapid regulation of the LPL gene expression at a transcriptional level. Ob1771 cells should be helpful in gaining some insights in the promoter function of the LPL gene and the *trans*-acting factors involved in its regulation. —**Pradines-Figuères, A., S. Barcellini-Couget, C. Dani, C. Vannier, and G. Ailhaud.** Transcriptional control of the expression of lipoprotein lipase gene by growth hormone in preadipocyte Ob1771 cells. *J. Lipid Res.* 1990. **31:** 1283–1291.

Supplementary key words 4β-phorbol-12-myristate 13-acetate • LPL mRNA • protein kinase C

Lipoprotein lipase (LPL) is synthesized in mouse adipose cells as a glycoprotein of M_r = 58,000 bearing two N-oligosaccharide side chains (1). Once secreted as active homodimer, the enzyme plays a key role by hydrolyzing, at the cell surface of capillary endothelium, plasma triglycerides from apolipoprotein C-II-containing lipoproteins (2). Numerous studies performed on intact fat pads as well as on isolated adipocytes have documented the role of hormones in the control of LPL synthesis and secretion in these cells (3). In this respect, insulin and corticosteroids are now recognized to stimulate the synthesis of the enzyme (4–6), whereas catecholamines seem to be involved, as are other lipolytic agents, in the acceleration of its degradative process (7, 8). The role of growth hormone (GH) in the regulation of LPL expression is poorly documented. It has been reported that, during differentiation of Ob17 preadipocyte cells, the emergence of LPL is coupled to growth arrest (9–11) and that GH increases moderately (<2-fold) the steady-state activity of LPL (12). However, a potent GH dependency for the expression of LPL activity (>6-fold) had been previously observed when differentiating Ob17 cells were exposed to bovine plasma instead of bovine serum (13). On the one hand, LPL mRNA levels in cells of the monocytic leukemia line THP-1 appear to be under the control of signals that activate protein kinase C (14). On the other hand, GH stimulates c-fos gene expression in Ob1771 by means of protein kinase C (15), and this phenomenon takes place at a transcriptional level (C. Dani and A. Doglio, unpublished results). Therefore, a possible role of GH upon the regulation of LPL gene expression, LPL synthesis and/or LPL secretion was more thoroughly investigated with respect to protein kinase C activation. So far, protein processing mechanisms have been shown to be important for the control of LPL expression, and recent studies have revealed that changes in LPL activity are mediated by

Abbreviations: LPL, lipoprotein lipase, GH, growth hormone; DME, Dulbecco's modified Eagle's medium; T_3, triiodothyronine; PBS, phosphate-buffered saline; H7, 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine; PMA, 4-β-phorbol-12-myristate 13-acetate; βPDco2, 4β-phorbol-12,13-didecanoate; αPDco2, 4α-phorbol-12,13-didecanoate; GAPDH, glyceraldehyde phosphate dehydrogenase.

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post-transcriptional mechanisms (1, 16, 17). In this study we report for the first time that, under appropriate conditions, GH rapidly stimulates LPL synthesis at a transcriptional level and that the effect of GH is mediated, at least in part, by means of activation of protein kinase C in preadipocyte Ob1771 cells.

MATERIALS AND METHODS

Cell culture

Ob1771 cells were plated at a density of 2×10^3 cells per cm^2 and grown in Dulbecco's modified Eagle's (DME) medium supplemented with 8% bovine serum, 200 units/ml of penicillin, 50 μg/ml of streptomycin, 33 μM biotin, and 17 μM pantothenate (18). This medium is termed standard medium. At confluence (day 0), which usually occurred within 5 days after seeding, the cells were exposed to standard medium supplemented with 17 nM insulin and 2 nM triiodothyronine (T_3). This medium was changed every other day. The emergence of LPL was observed between day 0 and day 2 after confluence. The cells were used at day 5. Unless otherwise stated, 15 h before performing the experiments, the medium was removed and replaced by DME medium containing 0.1% bovine serum (v/v), 17 nM insulin, and 2 nM T_3. This medium will be termed incubation medium.

Preparation of cell lysates

At the end of experiments, cells were washed twice at 4°C with phosphate-buffered saline (PBS) pH 7.4, solubilized during 30 min at 4°C in 5 mM sodium barbital buffer, pH 7.4, 1 M glycerol, and 150 mM NaCl (buffer A) containing 0.2% (w/v) Triton X-114 and 3 μg/ml heparin. Cell lysates could then be stored at −20°C. Before assaying LPL activity, cell lysates were treated at 30°C to pellet Triton X-114 micelles (19). Cell lysates used for quantitative immunoprecipitation were prepared as described below.

Labeling procedures

The labeling procedure was carried out in 1 ml of methionine-free DME medium containing 0.5% (v/v) dialyzed bovine serum. Before labeling, cells were washed twice and incubated for 15 min in the methionine-free DME medium for equilibration. The cells (approximately 8×10^5 cells per 60-mm dish) were then labeled in the same medium in the presence of 100 μCi of [^{35}S]methionine for 2 h. The labeling procedure was terminated by medium removal followed by two washes with ice-cold phosphate-buffered saline, pH 7.4.

Immunoprecipitation assays

To immunoprecipitate LPL quantitatively, antibodies raised in rabbit against mouse LPL were used. The characterization and specificity of this antibody preparation have been reported elsewhere (17). After labeling, cells were lysed at 0°C for 30 min in 20 mM sodium phosphate buffer, pH 7.3, 150 mM NaCl, 1 M glycerol (buffer B) containing 5 mM EDTA, 1% (w/v) Triton X-114, and a cocktail of protease inhibitors (0.1 mM PMSF, 4 μg/ml pepstatin, 4 mg/ml aprotinin). To pellet insoluble material, cell lysates were centrifuged at 12,000 *g* for 15 min. The supernatant was diluted 2-fold in buffer B containing 5 mM EDTA. The complete immunoprecipitation was ensured by an excess of antiserum; typically, 1 μl of antiserum was sufficient to precipitate 4 mU of LPL (~12 ng). LPL antigen was immunoprecipitated from Ob1771 cell lysates by adding the antiserum at a 1:100 dilution and incubating at 4°C overnight. A 1:1 slurry of Protein-A-Sepharose in buffer B containing 0.5% (w/v) Triton X-100 and 2 mM EDTA was added. The incubation was performed for 2 h at 4°C with end-over-end rotation of the tube. The beads were then washed three times in buffer B containing 0.5% (w/v) Triton X-100 and 0.2 mM EDTA, then twice with PBS containing 0.2% (w/v) gelatin. The beads were centrifuged at 2,000 *g* for 5 min and used for direct determination of the incorporated radioactivity (17).

Enzymatic assay of LPL

The activity is taken as the apoC-II-dependent hydrolysis of tri-[9,10-^{3}H]oleoyl glycerol (370 TBq/mol). Experimental conditions for the enzyme assay have been previously described (20).

When the secretion media were assayed for LPL activity, the media were first diluted 1.5-fold with ice-cold buffer A and maintained at 0°C in order to prevent any enzyme inactivation before assays. The values obtained for secreted activity had to be corrected for inactivation taking place during the period of secretion, as already reported (17, 21).

RNA analysis

RNAs were prepared by the guanidinium thiocyanate technique, as previously described (22). For Northern-blot analysis, 20 μg of total RNAs was electrophoresed on 1.2% (w/v) agarose gels, transferred onto a Hybond-N membrane (Amersham), and hybridized with nick-translated ^{32}P-labeled LPL probes: the first LPL cDNA probe (2.2 kb) was derived by antibody screening from poly(A)$^+$ RNAs of guinea pig adipocytes (23) and the second one (1.43 kb) from poly(A)$^+$ RNAs of mouse macrophages (24). RNAs (3.3 and 3.7 kb species) were quantitated by densitometry with an LKB-XL laser photodensitometer and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA signals (1.4 kb).

Nuclear transcription assays

Isolation of nuclei from Ob1771 cells and nuclear transcription assays were performed as described previously (12) with the following modifications: Ob1771 cells (10^7 cells per condition) were suspended in 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, and 0.5% (v/v) Nonidet P-40 (buffer C) and homogenized by gentle agitation. Nuclei were pelleted at 1000 *g* and washed once in buffer C and once in buffer C without Nonidet P-40. The nuclear pellets were resuspended in 100 μl 50 mM Tris-HCl, pH 8, 40% (v/v) glycerol, 5 mM $MgCl_2$, and 0.1 mM EDTA and then frozen in liquid N_2. The nuclei were thawed for run-off transcription and mixed and 100 μl reaction buffer (10 mM Tris-HCl, pH 7.4, 5 mM ($MgCl_2$, 300 mM KCl, 0.5 mM each of ATP, CTP, GTP, and 100 μCi of [α-^{32}P]UTP (800 Ci mmol^{-1}) and reacted at 30°C for 30 min. The reaction mixture was then treated with DNAse I (60 μg/ml) for 10 min at 30°C, deproteinized by digestion with proteinase K (50 μg/ml) after addition of 10 mM Tris-HCl pH 7.4, 10 mM EDTA, and 0.5% SDS, followed by phenol–chloroform extraction. RNA was rapidly precipitated from the aqueous phase with 2 M NH_4Ac and 2 volumes of ethanol. This step was repeated twice. Prior to hybridization the ^{32}P-labeled RNA were partially degraded with 0.1 M NaOH for 10 min on ice, neutralized by addition of 0.15 M HEPES, pH 6.4, and precipitated with ethanol.

Immobilization of DNA plasmids and hybridization

DNA from pGEM-4 control plasmid and plasmids containing mouse cDNA LPL (24) and rat cDNA GAPDH (25) were linearized by Eco-R1 restriction enzyme digestion, boiled, and blotted (3 μg of DNA/blot) onto nitrocellulose membrane. These membranes were dried at 80°C for 2 h. α-^{32}P-labeled nuclear RNAs (8×10^6 cpm) isolated from nuclear transcription experiments were used in 1 ml of hybridization buffer, hybridized to the filters for 48 h at 64°C, and washed as described (26). Filters were exposed to Kodak XAR-2 film at −80°C.

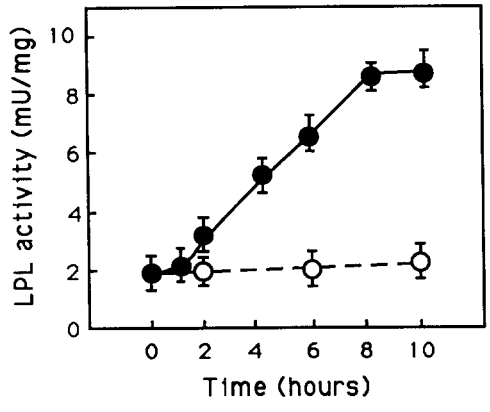

Fig. 1. Short-term stimulation of the expression of LPL activity by growth hormone. Ob1771 cells were maintained at 37°C in the incubation medium (see Materials and methods for details) supplemented (●) or not (○) from time zero with 4 nM GH. Determination of LPL activity was carried out in triplicate; error bars are ± SD. The data are representative of three independent experiments performed on different series of cells.

Materials

Culture media were obtained from Gibco (Cergy-Pontoise, France). Recombinant human growth hormone was a kind gift from KabiVitrum (Stockholm, Sweden). [α-^{32}P]UTP, [α-^{32}P]d-CTP, and nick translation kit were purchased from Amersham (France) and Protein-A–Sepharose from Pharmacia (France). Bovine serum and all other chemical products were obtained from Sigma (France).

RESULTS

Short-term and long-term stimulation of the expression of LPL activity by growth hormone

LPL was determined by a method that allows for the assay of the total cellular activity (17). As shown in **Fig. 1**, LPL was expressed at a detectable level in early confluent Ob1771 cells exposed to DME medium containing 0.1% bovine serum, 2 nM T_3, and 17 nM insulin. Addition of GH, at a concentration of 4 nM required in Ob1771 cells to saturate GH receptors (27) and to activate maximally the expression of c-fos and IGF-I genes (15, 22), led within a couple of hours to a 1.5-fold increase of LPL cellular activity, and to a 4.5-fold increase within 8 h. The presence of insulin was not required to observe a stimulatory effect of GH as, in another series of experiments, the stimulation factor varied from 2.4 to 3.6 when cells were exposed and maintained in various concentrations of insulin (0 to 17 nM) for 15 h before GH addition; whereas it varied from 2.9 to 3.5 when cells, previously maintained in the presence of 17 nM insulin, were then exposed from 0 to 17 nM insulin at the time of GH addition. The expression of both cellular and secreted activities was stimulated by GH (**Fig. 2**). The maximal stimulation of cellular activity occurred within 24 h and that of secreted activity within 6 h. The stimulation factor brought about by GH was higher for the secreted than for the cellular enzyme (18-fold vs 6-fold). The dose-response relationships of added GH to the activity of cellular and secreted LPL, determined after an 8-h exposure to the hormone, are presented in **Fig. 3**. The expression of cellular and secreted LPL activities was near maximum at 4 nM whereas the concentrations required for half-maximal effect were 0.4 nM for the cellular enzyme and 0.8 nM for the secreted enzyme. These values are in good agreement with the K_d value of ~1 nM reported for GH receptor sites in Ob1771 cells (27). It must be stressed in this respect that GH receptors are present both in growing, nondifferentiated and resting,

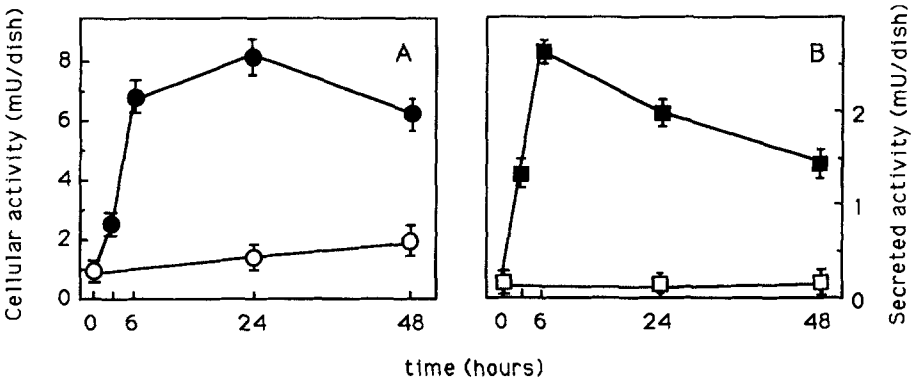

Fig. 2. Long-term stimulation of the expression of cellular and secreted LPL activities by growth hormone. Ob1771 cells were maintained at 37°C in the incubation medium (see Materials and Methods for details) supplemented (●, ■) or not (○, □) from time zero with 4 nM GH. At the indicated times, cell lysates were prepared in duplicate for the determination of cellular LPL activity (○, ●). Duplicate secretion media were obtained in parallel by incubating intact Ob1771 cells, previously exposed to 4 nM GH, in the presence of fresh incubation medium containing 3 μg/ml of heparin. After 30 min at 37°C, media were collected for LPL assays (□, ■). Determination of cellular and secreted LPL activities was carried out in duplicate; errors bars are ± SD. The average protein content per dish was 0.45 ± 0.05 mg. The data are representative of two independent experiments performed on different series of cells.

differentiating Ob1771 cells that were or were not exposed to GH (27). Once again, the secretion of LPL from GH-deprived cells were very low and the stimulation factor observed upon GH addition was higher for the secreted than for the cellular enzyme. Taken together, the results of Figs. 1–3 show that growth hormone stimulates the expression of LPL quite rapidly. This phenomenon is specific for cells expressing the differentiation program since growing, nondifferentiated Ob1771 cells did not express LPL after GH addition. In contrast, when the differentiation of dispersed Ob1771 cells, which were exponentially growing, was triggered by growth arrest using a thymidine block (9), a stimulation of LPL activity (>2-fold) could be observed after GH addition (A. Pradines-Figuères, unpublished results). Among hypotheses to explain the effect of GH, a general stimulation of protein synthesis could be ruled out. It was observed in Ob1771 cells, in contrast to isolated hemidiaphragm of hypophysectomized rats (28), that [^{14}C]leucine incorporation into proteins was identical in GH-deprived and GH-supplemented cells. The possibility that GH could act by decreasing the degradation rate of active LPL could also be ruled out since the half-life of LPL activity in cycloheximide-treated cells (20) previously exposed for 24 h to 4 nM GH was identical whether or not GH was removed from the incubation medium ($t_{1/2}$ ~35 min). Therefore, experiments were undertaken to study the possible effects of GH on the main steps leading to the acquisition of active LPL.

Parallel increases of LPL mRNA, LPL synthesis, and LPL activity in GH-supplemented cells

Ob1771 cells were exposed to GH and parallel estimates were made as a function of time with respect to LPL mRNA content, LPL protein, and LPL activity. The curves of **Fig. 4A** indicate a parallel increase in LPL protein and LPL activity, excluding a change in the catalytic efficiency of the enzyme upon GH addition. Moreover, the curves clearly show that the increase of LPL protein can be entirely accounted for by an increase of LPL mRNA content, excluding an effect of GH upon the translational and post-translational steps leading to active enzyme. It should be pointed out that both LPL and mRNA species (3.3 and 3.7 kb) increased in parallel after

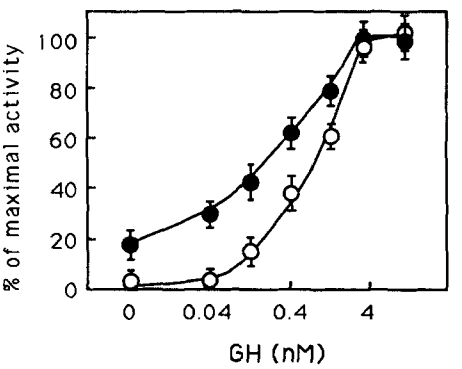

Fig. 3. Dose-response curve of cellular and secreted LPL activities to GH concentrations. Cells were maintained at 37°C in the incubation medium supplemented with varying concentrations of GH as indicated. After 8 h, cellular LPL activities (●) were determined in duplicate as described in Fig. 2. Secreted LPL activities (○) were determined in triplicate assays from secretion media obtained in parallel by incubating intact Ob1771 cells, previously exposed to various concentrations of GH, in the presence of fresh incubation medium containing 3 μg/ml of heparin. After 30 min at 37°C, media were collected for LPL assays. The results (means ± SD) are expressed as percent of the maximum activity values obtained for cells maintained in the presence of 12 nM GH. The data are representative of three independent experiments performed on different series of cells.

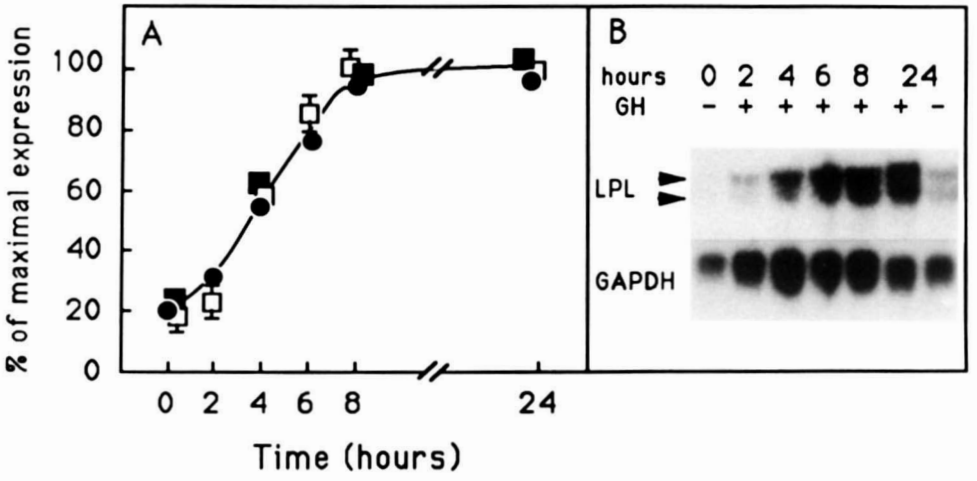

Fig. 4. Comparative effect of GH on the expression of LPL mRNAs, LPL protein and LPL activity. Ob1771 cells were maintained at 37°C in the incubation medium (see Materials and Methods for details) supplemented at time zero with 4 nM GH. At the indicated times, total RNAs were isolated from four pooled 100-mm dishes, electrophoresed, and probed for LPL mRNAs (3.3 kb plus 3.7 kb species) as described in Materials and Methods (●). Cellular LPL activity was determined in duplicate (means ± SD) for two 35-mm dishes (□). For the determination of LPL protein, cells in 60-mm dishes were labeled for 2 h prior to the indicated times in the presence of [^{35}S]methionine. Cell lysates were prepared and ^{35}S-labeled LPL was determined as previously described (17) (■). A: The results are expressed as percent of the maximum values obtained after 24 h exposure to GH. One hundred percent corresponds to LPL activity of 16 mU/mg of cell protein, LPL protein to 15, 217 dpm/mg of cell protein, and LPL mRNAs (3.3 plus 3.7 kb species) to an arbitrary unit obtained by densitometric scanning of autoradiographs of Northern-blot hybridization and normalization with GAPDH mRNA as internal control. B: Northern-blot analysis of LPL mRNAs of cells exposed or not to 4 nM GH for the indicated times. The arrows indicate 3.3 and 3.7 LPL mRNA species.

GH addition (Fig. 4B), excluding that GH was favoring the preferential use of one of the alternate polyadenylation signals (23, 24) and/or the preferential stabilization of one of the two LPL mRNA species. It is of interest to observe that, among the early markers of adipose cell differentiation so far characterized, the stimulation by GH appears rather unique to the LPL gene as the expression of pOb24 gene remains unaffected upon hormone addition (not shown). Since a significant increase of LPL mRNA content was observed within 2 to 4 h after GH addition, as reported previously for the expression of IGF-I gene in Ob1771 cells (22), it was assumed that the regulation of LPL gene expression was also taking place primarily at a transcriptional level. This point was next examined.

The relative transcription rate of the LPL gene was measured using nuclear transcription assay (29). As shown in **Fig. 5**, when 5-day post-confluent cells were exposed for 15 h to 4 nM GH, the transcription rate of LPL gene increased 5.6-fold, in agreement with the 5.2-fold increase of LPL activity, whereas no transcription activity of LPL gene was detected in exponentially growing cells. The transcription signal obtained with the GAPDH gene remained unchanged under all conditions. Taken together, the results of Fig. 5 indicate that the transcription rate of LPL gene is primarily responsible for the rapid accumulation of LPL mRNAs and increase of LPL activity during exposure to GH. The effect of GH removal, following exposure of Ob1771 cells to the hormone for 24 h, was next investigated. The results of **Fig. 6** show that Ob1771 cells respond to GH in a reversible manner. A rapid and parallel disappearance of both LPL mRNA species occurred. Both species of 3.7 and 3.3 kb decreased within 4 h to basal values obtained in untreated cells before exposure to GH. This result is similar to that obtained for the steady-state level of the various species of IGF-I mRNAs (22).

Protein kinase C-mediated effect of GH upon LPL gene expression

A remarkable property of the c-fos gene is that its rapid induction by GH appears to be mediated in Ob1771 cells by means of the activation of protein kinase C (15). So far, the regulation of the various genes responding to GH in Ob1771 cells was found to occur in a reproducible manner at a transcriptional level (12, 22 and C. Dani and A. Doglio, unpublished results). Since activation of protein kinase C was separately shown to be critical for the expression of LPL mRNAs in THP-1 cells, a monocytic leukemia cell line (14), the direct activation of protein kinase C was compared to the effect of GH on the regulation of LPL gene in Ob1771 cells. The results of **Fig. 7** show that exposure to GH for 8 h led to a 7-fold increase

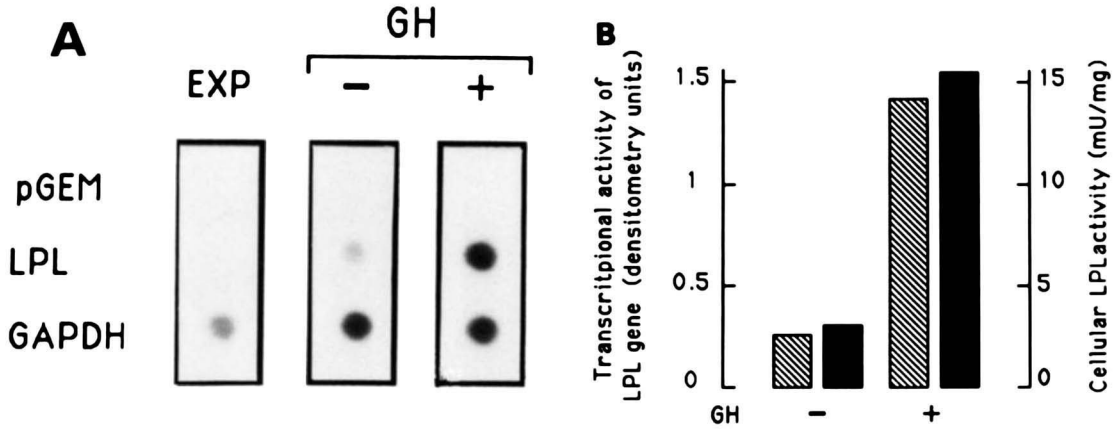

Fig. 5. Effect of growth hormone on the transcriptional activity of LPL gene. Ob1771 cells were exposed for 15 h at 37°C to the incubation medium in the absence (–) or in the presence (+) of 4 nM growth hormone. Nuclei were isolated and [α-^{32}P]UTP was incorporated into nascent RNA chains as described in Materials and Methods. Labeled RNAs were then hydridized to the DNAs immobilized onto nitrocellulose filters. After treatment with the RNAses the filters were washed and exposed for 3 days to X-ray film. Analysis of the transcriptional activity of exponentially growing Ob1771 cells were performed in parallel (EXP) (A). The values obtained by densitometry were normalized to GAPDH signals (hatched bars). Cellular LPL activity was determined as described in Fig. 4 and Materials and Methods (stippled bars) (B).

in the level of LPL mRNAs (Fig. 7A). The expression of LPL gene was partially abolished (~56%) by the kinase inhibitor (1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7) (30). 4β-Phorbol-12-myristate 13-acetate (PMA) at an optimal concentration of 3 nM (first assayed between 0.1 and 300 nM) was active in causing LPL mRNA accumulation up to 50% of that obtained in the presence of GH; in that case, H7 was able to abolish completely the effect of PMA. It is of interest that LPL mRNA accumulation (Fig. 7A) and LPL activity (Fig. 7B) appeared not to be affected by H7 in GH-deprived cells, excluding some unspecific effects of this inhibitor on LPL gene expression. The results obtained for LPL activity were very similar to those obtained for LPL mRNAs. The activity was increased approximately 5-fold in the presence of GH and prior addition of H7 lowered the stimulation by 50% (Fig. 7B). Exposure of the cells to PMA led to a stimulation that was half of that obtained with GH and this stimulation was completely abolished by H7. Among other putative agents activating protein kinase C, 4β-phorbol-12,13-didecanoate (3 nM) was effective whereas, as expected, 4α-phorbol-12,13-didecanoate (3 nM) was ineffective. Similar if not identical results were obtained for the secreted LPL activity as compared to the cellular activity (A. Pradines-Figuères, unpublished results). Taken together, the results of Fig. 7 indicate that the regulation of LPL gene expression by GH is partly mediated by activation of protein kinase C and that the inhibitor H7 can abolish this part only. To ascertain that protein kinase C was playing a role in the regulation of LPL gene expression, other inhibitors of the enzyme were used. The results of **Table 1** indicate clearly that the effect of GH was abolished by half by polymixin B, staurosporin, and sphingosine, in a way quite similar to that observed with H7, the maximal inhibitory effect of sphingosine being already observed at a concentration of 25 μM.

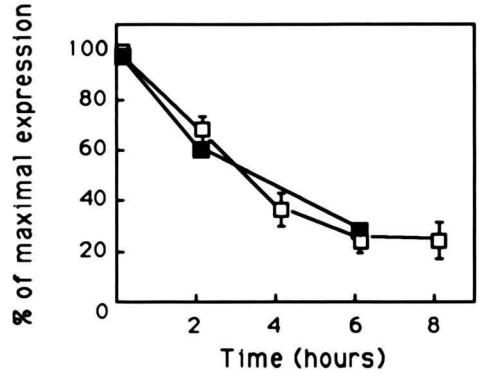

Fig. 6. Changes in the expression of LPL mRNA levels and cellular LPL activity following GH removal. At day 4 post-confluence, Ob1771 cells were exposed at 37°C to the incubation medium supplemented with 4 nM GH. Twenty four h later, cells were rinsed at 37°C with the incubation medium: three times with 10 ml for 5 min and once with 10 ml for 10 min per 100-mm dish (or using 2 ml volumes per 35-mm dish). Cells were then incubated from time zero in the incubation medium deprived of GH. At the times indicated, four pooled 100-mm dishes and duplicate 35-mm dishes were used for the determination of LPL mRNA content (■) and cellular LPL activity (□) (error bars correspond to ± SD), respectively. The results are expressed as percent of the initial values obtained at time zero (arbitary units for LPL mRNAs and 7.53 mU/mg of cell protein for the cellular LPL activity).

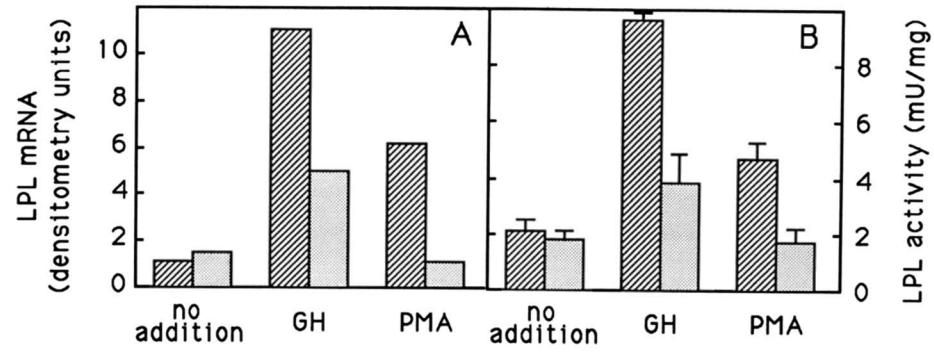

Fig. 7. Induction of LPL mRNAs and cellular LPL activity mediated by protein kinase C. Ob1771 cells were maintained for 8 h at 37°C in the incubation medium supplemented or not with 4 nM GH or 3 nM PMA, in the presence (stippled bars) or absence (hatched bars) of 100 μM H7. H7 was added 30 min before exposure of the cells to the various effectors. After incubation, LPL mRNAs (A) were quantitated from four pooled 100-mm dishes, whereas cellular LPL activity (B) was determined from duplicate 35-mm dishes; error bars correspond to ± SD.

DISCUSSION

Growth hormone, apparently acting through its own cell receptors, regulates the expression of the LPL gene in preadipose Ob1771 cells quite rapidly. The preadipose cells responding to GH stimulation are committed, i.e., are already expressing the early part of the differentiation program. The detailed mechanism by which GH stimulates LPL mRNA accumulation is not known, but clearly the regulation of LPL gene expression takes place, primarily at a transcriptional level, in a way similar to other GH-responsive genes, namely c-fos, IGF-I, and GPDH genes (12, 15, 22). However, in the case of the LPL gene only, GH plays only the role of a modulating hormone but not that of an obligatory hormone since LPL mRNA, LPL protein, and LPL activity are expressed to some extent in the absence of GH (Fig. 4). Once activated, the transcription of the LPL gene requires the presence of GH (Fig. 6). The present study has shown that, in Ob1771 cells, the action of GH is only mediated in part by means of protein kinase C activated by diacylglycerol (15). The situation of the LPL gene is therefore intermediary between that of the c-fos gene (activated by means of the activation of protein kinase C) and that of the IGF-I gene (activated by means of a mechanism different from the activation of protein kinase C). Growth hormone has been shown to trigger diacylglycerol production but not the release of inositol phosphates (15); in agreement with this observation, no change in the concentration of intracellular calcium could be detected by using Indo-I as fluorescent probe. This lack of calcium mobilization agrees with the fact that neither the calcium ionophore A23187 in the absence of GH, nor the calmodulin antagonist N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) (31) have any effect on the cellular LPL activity in the presence of GH (A. Pradines-Figuères, unpublished results). Since one of the transduction signals of GH action is the formation of diacylglycerol associated with a phospholipase C-mediated hydrolysis of glycerophospholipids other than inositol phospholipids (15), it is suggested that, as for the activation of IGF-I gene, either the water-soluble product(s) arising from hydrolysis of these unidentified complex lipids or a transduction mechanism of GH separate and unrelated to glycerophospholipid hydrolysis are involved in the more potent stimulatory effect of GH as compared to phorbol esters.

It is of interest to observe that the higher stimulation factor brought about by GH for the secreted enzyme compared with the cellular enzyme is apparently due to the

TABLE 1. Effect of various inhibitors of protein kinase C on cellular LPL activity

Culture Conditions	Cellular LPL Activity: No Addition	Cellular LPL Activity: Plus Growth Hormone (4 nM)
	mU/mg of cell protein	
Experiment 1		
No drug addition	4.1 ± 0.4	14.36 ± 0.64
H7 (100 μM)	4.48 ± 0.22	6.19 ± 0.45
Staurosporin (2 nM)	4.2 ± 0.36	8.58 ± 0.6
Polymixin B (500 μg/ml)	4.4 ± 0.24	8.6 ± 0.43
Experiment 2		
No drug addition	1.69 ± 0.13	13.15 ± 0.33
H7 (100 μM)	1.74 ± 0.2	6.84 ± 0.6
Polymixin B (500 μg/ml)	1.91 ± 0.13	6.52 ± 0.94
Experiment 3		
No drug addition	2.22 ± 0.04	16.43 ± 0.25
Sphingosine (2.5 μM)	1.59 ± 0.17	12.06 ± 0.12
Sphingosine (25 μM)	2.06 ± 0.13	7.11 ± 0.2
Sphingosine (200 μM)	2.67 ± 0.18	6.59 ± 0.18

Ob1771 cells were maintained for 8 h at 37°C in the incubation medium supplemented or not as indicated. Determination of cellular LPL activities was performed on duplicate 35-mm dishes; mean values ± SD are shown.

absence of LPL secretion, but not to that of LPL synthesis in GH-deprived cells. Thus it cannot be excluded that GH may have additional and distinct effects on enzyme secretion. If it were so, this situation would be reminiscent of that observed for insulin, which appears to stimulate LPL secretion (32) independently of its stimulatory effect on LPL synthesis (4).

The expression of the LPL gene is clearly a differentiation-dependent event and the regulation of its expression by GH deserves some comment, as it had been reported (11) and confirmed in this study that GH is not required for the emergence of LPL. It had also been reported that GH moderately increases (<2-fold) the activity level of LPL in the presence of 8% bovine serum; it appeared that lowering the serum concentration is critical to observe a potent stimulatory effect of GH. This observation is in agreement with the characterization of various hormones and growth factors from bovine serum that have been characterized as inhibitors of the expression of LPL gene in GH-supplemented cells (33).

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