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Induction of prolactin expression and release in human preadipocytes by cAMP activating ligands

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Abstract

In addition to the pituitary, prolactin (PRL) in humans is produced at non-pituitary sites where it acts as a cytokine. We previously reported that PRL is expressed and released from breast adipose explants, raising the question as to the dynamics of its production and its regulation. Preadipocytes were isolated from breast adipose tissue obtained during breast reduction. PRL expression was transiently increased during early preadipocyte differentiation. Both isoproterenol, a β-adrenergic receptor agonist, and PACAP, pituitary adenylate cyclase activating peptide, increased PRL expression, and release from preadipocytes. This stimulation was suppressed by several protein kinase inhibitors, suggesting involvement of multiple signaling pathways. Transfection of preadipocytes with a superdistal PRL promoter/luciferase reporter revealed two stimulatory domains and an inhibitory domain. These data establish the transcriptional regulation of adipocyte PRL by the superdistal PRL promoter, its transient expression during adipogenesis, and the stimulatory effect of catecholamines and PACAP.

Keywords: Prolactin; Breast preadipocytes; Differentiation; IBMX; Isoproterenol; PACAP; Kinase inhibitors; Superdistal prolactin promoter

Prolactin (PRL) is best known as a 23 kDa pleiotropic hormone of pituitary origin. Unlike other species, PRL in humans is also produced in non-pituitary sites, including the decidua, lymphocytes, prostate, and breast [1]. Thus, in addition to acting as a circulating hormone, human PRL (hPRL) is classified as a cytokine, given its multiple production sites and ubiquitous receptor (PRL-R) distribution [2]. Although pituitary and non-pituitary sites produce an identical PRL protein, the regulation of its transcription is dissimilar. Pituitary PRL is driven by a proximal promoter that requires Pit-1 transcription factor for activation, is inhibited by dopamine and is stimulated by neuropeptides and estrogens [3]. In contrast, non-pituitary PRL is regulated by a superdistal promoter, located 5.8 kb upstream of the pituitary start site [4], which is unresponsive to dopamine or estrogen. Previous studies have reported that cAMP activators stimulate PRL expression in decidual cells [5,6] and Jurkat lymphocytes [7], but the natural ligands that regulate PRL are unknown and likely differ among the various PRL-producing sites.

Using RT-PCR and a sensitive bioassay for PRL, we recently found that human breast adipose explants produced significant amounts of PRL [8]. Neither estrogen nor progesterone affected PRL release from these explants. PRL is a potent stimulator of signal transduction and activator of transcription (STAT 5a/b), whose expression is induced during differentiation of 3T3-L1 adipocytes [9] and human subcutaneous preadipocytes [10]. Exogenous PRL augments adipogenic conversion in NIH-3T3 cells [11], and PRL can replace the requirement for fetal bovine serum (FBS) for adipogenesis in 3T3-L1 cells [12]. Notably, murine preadipocytes depend on FBS, which contains significant amounts of bovine PRL and placental lactogens [13], for an effective adipogenesis [14], whereas human preadipocytes differentiate better under serum-free conditions [15].

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Given that PRL is produced by human adipose tissue and appears to be involved in the process of adipogenesis, we questioned whether it is expressed by human preadipocytes and if so, whether its expression is altered during differentiation. In addition, we opted to identify ligands that affect PRL expression and release from preadipocytes and determine whether PRL transactivation in adipocytes is driven by the superdistal PRL promoter.

Materials and methods

Subjects. Breast adipose tissue was obtained from 40 women undergoing breast reduction surgery. Of these, 68% were Caucasians and 32% were African-American women. The range of ages was from 18 years to 77 years with the average age of 36.5 years. Patients with diseases of the breast were excluded from the study. All samples were obtained using informed patient consent approved by the Institutional Review Boards (IRB) of the University of Cincinnati and The Christ Hospital, Cincinnati.

Cell isolation and culture. Breast adipose tissue was cut into small pieces and then incubated for 30–45 min at 37 °C in Hanks' Balanced Salt Solution containing 200 U/ml of collagenase type I (Worthington, Lakewood NJ). After centrifugation at 1000*g* for 1 min, floating adipocytes were removed and the preadipocyte fraction was filtered through a 70 μm nylon mesh (BD Biosciences, San Jose, CA), and centrifuged at 1500*g* for 10 min. Cells were suspended in erythrocyte lysis buffer (154 mM ammonium chloride, 10 mM potassium carbonate, and 0.1 mM EDTA), repelleted, and plated in DMEM/F12 with 10% FBS (Gibco). Preadipocytes were cultured for 5–6 days and then stored in liquid nitrogen.

Preadipocyte differentiation. Preadipocytes pooled from 3–4 patients were plated at 3.7×10^4 cells/cm^2 in 6-well plates in DMEM/F12 with 10% FBS. After 24 h, cells were incubated in serum-free DMEM/F12 for 24 h and then incubated for three days in a differentiation medium composed of serum-free DMEM/F12 with 200 μM 3-isobutyl-1-methylxanthine (IBMX; Sigma), 1 μM dexamethasone (Biomol, Plymouth Meeting, PA), 33 μM D-biotin (Sigma), 17 μM D-pantothenic acid (Sigma), 10 μg/ml transferrin, 1 nM D-triiodothyronine (Sigma), and 66 nM human insulin (Sigma). This was followed by incubation for additional 11 days in the same medium without IBMX. To verify morphological conversion, cells were incubated with 1 mg/ml oil red O (Sigma) in isopropanol for 5 min. After washing in 70% ethanol and counterstaining in Harris hematoxylin (Sigma), cells were imaged by microscopy.

Determination of gene expression by RT-PCR. In the first experiment, cells were harvested on days 0, 1, 3, 7, and 14 of differentiation. In the second experiment, preadipocytes were incubated for 24 h with individual components of the differentiation medium as well as with 20 μM of ciglitazone (Cayman Chemicals, Ann Arbor, MI), a peroxisome proliferator activating receptor-γ (PPAR-γ) agonist. In the third experiment, preadipocytes were incubated with 200 μM IBMX, 1 μM isoproterenol, or 10 nM PACAP-38 for 2, 8, 24, or 48 h. Total RNA was extracted using Tri Reagent (MRC, Cincinnati, OH), and 5 μg of RNA was reverse transcribed using random hexamers and Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA). PCR was performed on 250 ng of cDNA using intron-spanning primers for PRL, PRL-R, LPL, leptin, adiponectin, and S18 (Table 1). PCR were done on a thermocycler (Eppendorf, Westbury, NY), after determining the linear range for each primer set. The PCR conditions were: 94, 58, and 72 °C at 30 s each for 32 cycles (28 cycles for S18; 34 cycles for PRL). Products were separated on 2% agarose gel containing ethidium bromide and photographed.

PRL release from preadipocytes. Pooled preadipocytes (140,000 cells/well) were plated in 48-well plates in DMEM/F12 with 3% charcoal-stripped serum (CSS). In the first experiment, cells were incubated with IBMX (200 μM), isoproterenol (1 μM), or PACAP (10 nM), and conditioned media (CM) were collected after 24 or 48 h and analyzed for PRL by the Nb2 bioassay. In the second experiment, preadipocytes were pre-incubated with H89, a protein kinase A (PKA) inhibitor, LY294002, a phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) inhibitor, or UO126, a MEK inhibitor (each at 10 μM, all from Calbiochem, San Diego, CA). After 1 h, IBMX, PACAP or isoproterenol at the same concentrations as above were added. CM were collected after 24 h and analyzed for PRL.

Nb2 bioassay for PRL. Rat Nb2 lymphocytes were cultured as previously described [8]. Briefly, cells were plated in 96-well plates (20,000 cells/well) and incubated with human PRL (NIDDK) in triplicate and CM aliquots in duplicate. After three days, cell number was determined by the MTT method [8]. The amount of PRL in the CM was calculated from the standard curve, with a lowest detectable level of 2 pg/well. To verify assay specificity for PRL, Nb2 cells were co-incubated with CM, IgG-purified anti-hPRL antibodies (6 μg/well) or control rabbit IgG (6 μg/well). To examine whether Nb2 cells were directly affected by cAMP-activating ligands or protein kinase inhibitors, cells were incubated with increasing amounts of hPRL in the presence of each compound at a concentration calculated to be contained within CM aliquots. Modified standard curves were then used to calculate the PRL levels in the corresponding CM aliquots.

Metabolic labeling for de novo synthesis of PRL. Preadipocytes were incubated in DMEM/F12 with or without 200 μM IBMX. After 18 h, cells were incubated for 6 h with ^{35}S-labeled L-methionine and L-cysteine (ICN), as previously described [8]. After clearing with NRS (control) and immunoprecipitation, samples were separated on 12% SDS–PAGE, and the labeled proteins were visualized by autoradiography.

Table 1
Primer sets used in RT-PCR

Gene	Accession number	Sense (5′–3′) forward; antisense (5′–3′) reverse	Size (bp)
PRL	NM_000948	TCGTCCTGTCCCACTACATCCATA AGCAGAAAGGCGAGACTCTTCATC	434
PRL-R	NM_000949	GAGTCCAGCGACCTTCATTCAGAT GTTCTTCAGACTTGCCCTTCTCCA	220
Leptin	NM_000230	TCTTGTGGCTTTGGCCCTATCT CCAGTGTCTGGTCCATCTTGGATA	181
Adiponectin	NM_004797	AGGGTGAGAAAGGAGATCCAG GGGCATGTTGGGGATAGTAA	194
Lipoprotein lipase (LPL)	NM_000237	TGGAACTGCACCTGTAGGC GGGCATGTTGACATTTACCC	199
Ribosomal protein 18 (S18)	M10098	CGGCTACCACATCCAAGGAA GCTGGAATTACCGCGGCT	190

Determination of superdistal promoter activity. Decidual PRL (dPRL) promoter constructs, cloned into PGL3E luciferase reporter vector (Promega, San Luis Obispo, CA), were gifted by Dr. Anoop Brar, Children's Hospital, Cincinnati, OH. These included a −3000/+66 dPRL-pGL3E construct containing the full superdistal promoter sequence and the −2040, −1556, −675, −317, and −4 dPRL truncated constructs; pGL3E was used as a control. Construct structures were verified by sequencing. Preadipocytes were transfected using GenePorter2 (GTS, San Diego, CA). Gaussia luciferase pCDNA3 (Prolume, Pinetop, AZ) served as a transfection control. After 72 h, cells were lysed and analyzed for luciferase activity using TopCount luminometer (Packard Instruments, Downers Grove, IL). Transfections were normalized to Gaussia luciferase and expressed as relative luciferase activity vs empty vector (pGL3E).

Data Analysis. All experiments were repeated 2–3 times. When appropriate, results are expressed as means ± SEM. Data were analyzed by ANOVA or a two-tailed Student's t test. A value of $P < 0.05$ was considered significant.

Results

Transient induction of PRL expression during early preadipocyte differentiation

To verify lack of contamination of the preadipocyte preparation by endothelial cells, fluorescent-labeled DiI-Ac LDL, which is specifically taken up by endothelial cells, was used. Whereas bovine aortic endothelial cells (used as a positive control) had a uniform fluorescent labeling, the isolated preadipocytes showed very little labeling, indicating negligible contamination with endothelial cells (not shown). To induce adipogenesis, preadipocytes were treated with a common differentiation protocol [15]. Morphological conversion was verified by

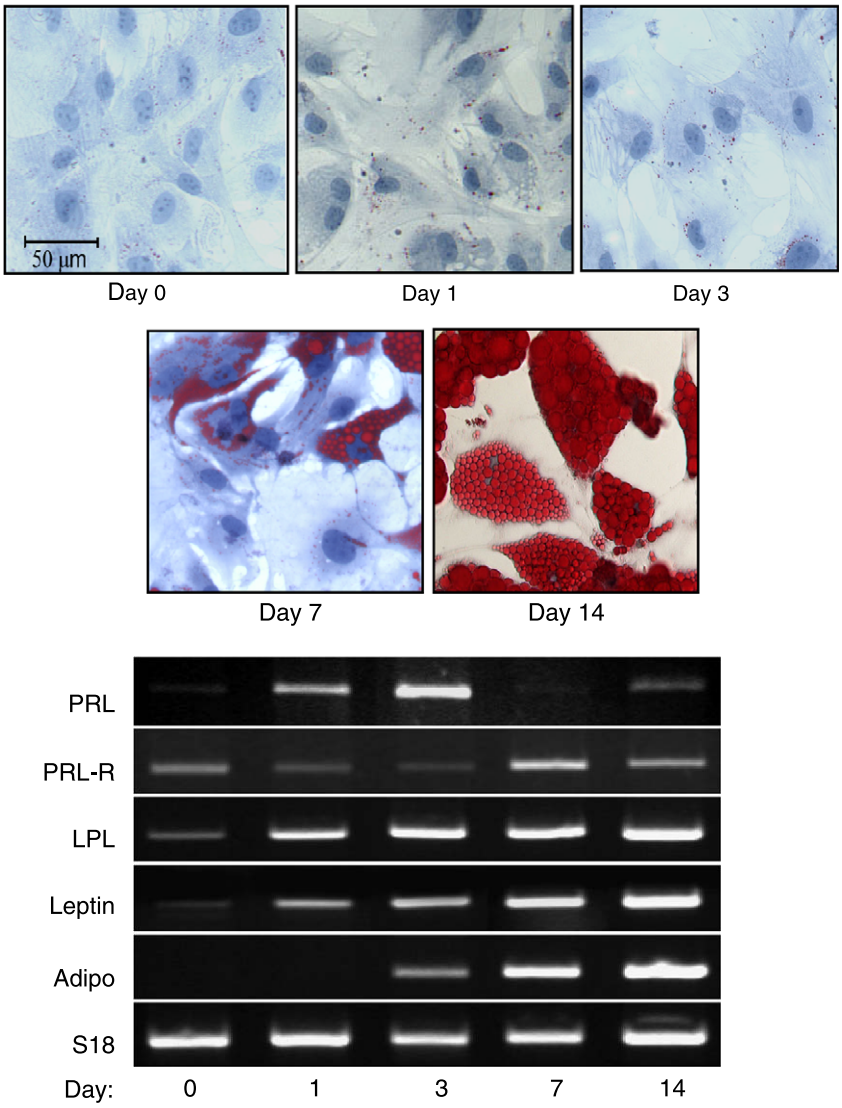

Fig. 1. Upper panels: morphological conversion of differentiating breast preadipocytes. Preadipocytes were treated with a differentiation medium as described in Materials and methods. Lipid accumulation is revealed by staining with oil red O. All photographs were taken at the same magnification. Lower panel: transient expression of PRL during an early differentiation phase. RNA, isolated on days 0, 1, 3, 7, and 14 of differentiation, was analyzed by RT-PCR for PRL, PRL-R, lipoprotein lipase (LPL), leptin, and adiponectin (adipo). S18 served as a control. Primer sequences and expected product sizes are detailed in Table 1.

lipid accumulation, as seen by staining with oil red O (Fig. 1, upper two panels). On day 0, preadipocytes appear fibroblast-like with elongated morphology, becoming more rounded on day 3. Lipid droplets were apparent on day 7, and by day 14, the majority of cells were greatly enlarged and contained numerous lipid droplets. PRL expression was very low on day 0, increased markedly on days 1 and 3 and reduced thereafter (Fig. 1, lower panel). PRL-R expression was low during the early differentiation phase, followed by an increase at later times. As expected, the adipocyte-specific genes LPL, leptin, and adiponectin increased progressively throughout adipogenesis.

Induction of PRL expression by cAMP activating ligands

Since PRL was induced during the first three days of adipogenesis, we question whether this was due to stimulation by a component of the differentiation medium. As shown in Fig. 2, upper panel, only IBMX, but not insulin, dexamethasone or ciglitazone, increased PRL mRNA levels. Since IBMX raises intracellular cAMP levels, we explored the effects of ligands which activate cAMP. After screening several cAMP-activating ligands whose receptors are known to be expressed in adipose tissue, we pursued isoproterenol, a β-adrenergic receptor agonist, and PACAP. Both ligands stimulated PRL gene expression within 8 h of incubation (Fig. 2, middle panel). Other compounds, including dibutyryl cAMP, epinephrine, and vasoactive intestinal polypeptide (VIP), also stimulated PRL gene expression (not shown).

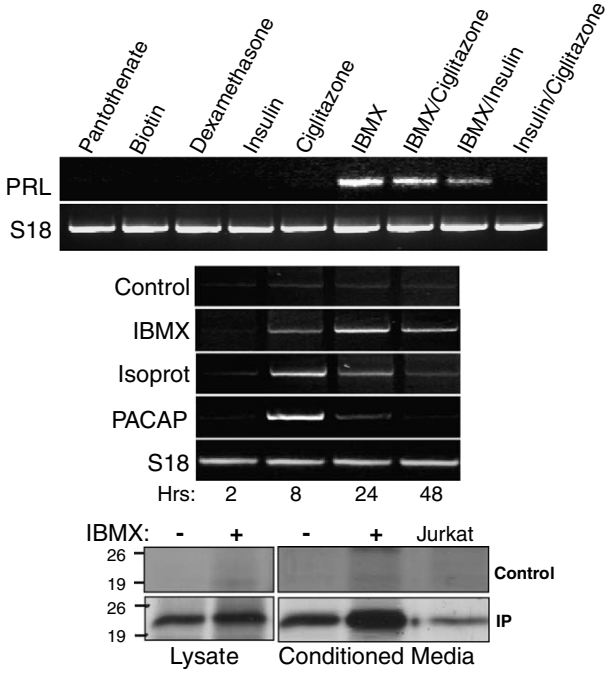

Fig. 2. Upper panel: induction of PRL expression in preadipocytes by IBMX. Preadipocytes were incubated with various components of the differentiation medium for 24 h. PRL expression, determined by RT-PCR, increased only in IBMX-treated cells. S18 served as a control. Middle panel: induction of PRL expression in preadipocytes by cAMP activators. Preadipocytes were incubated with 200 μM IBMX, 1 μM isoproterenol (isoprot), or 10 nM PACAP. After 2, 8, 24, and 48 h, PRL expression was determined by RT-PCR. S18 served as a control. Lower panel: de novo synthesis of PRL by preadipocytes. Cells were incubated with ^{35}S-labeled cysteine in the presence or absence of 200 μM IBMX, as described in Materials and methods. Following clearing with normal rabbit serum (control) or hPRL antiserum (IP), samples were separated on SDS–PAGE and autoradiographed. Jurkat cells served as a positive control.

De novo synthesis of PRL by preadipocytes

To verify de novo synthesis of PRL and confirm its molecular size, preadipocytes were subjected to metabolic labeling with ^{35}S-methionine followed by electrophoresis and autoradiography. Jurkat cells served as a positive control. Fig. 2, lower panel, shows the presence of ^{35}S-labeled 23 kDa PRL in both cell lysates and CM. Treatment with IBMX for 24 h caused a modest increase in PRL protein content in the cell lysates and a marked increase in the CM.

Sensitivity and specificity of the Nb2 bioassay

As shown in Fig. 3, upper left panel, Nb2 cell proliferation increased progressively in response to 2–150 pg of hPRL. Increasing CM aliquots from preadipocytes generated a proportional increase in Nb2 cell proliferation within the linear portion of the curve (not shown). Of the cAMP activators, only IBMX had a significant suppressive effect (20–25%) on the Nb2 proliferative response. Therefore, PRL levels in CM aliquots containing IBMX were calculated from a modified standard curve with IBMX. Co-incubation of Nb2 cells with anti-hPRL antibodies abolished the ability of CM to increase cell proliferation whereas the control IgGs had no effect (Fig. 3, upper right panel). This confirmed that PRL accounted for all of the detectable bioactivity in the CM.

Stimulation of PRL release from breast preadipocytes by isoproterenol and PACAP

To ascertain that not only PRL gene expression but also PRL release is stimulated by the cAMP activators, primary preadipocytes were incubated with isoproterenol, PACAP or IBMX and the CM analyzed for PRL release by the bioassay. As evident in Fig. 3, lower panel, PRL release was increased by the three compounds at both 24 and 48 h. Co-incubation of isoproterenol with IBMX was not more effective than IBMX alone.

Effect of protein kinase inhibitors on PRL release from preadipocytes

Although activation of adenylate cyclase is a common feature of all tested compounds, several signaling

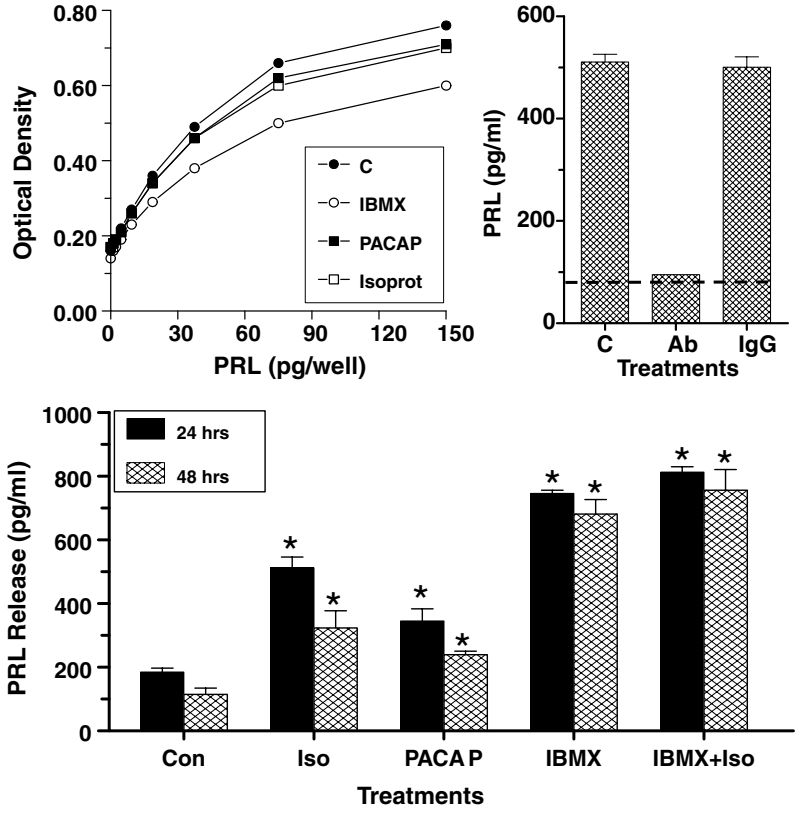

Fig. 3. Upper left panel: effect of cAMP activating ligands on Nb2 cell proliferation. Cells were incubated with increasing doses of hPRL in the absence (C) or presence of IBMX, PACAP or isoproterenol (isoprot) at concentrations calculated to be contained in CM aliquots. Only IBMX has a significant ($P < 0.05$) suppressive effect on the Nb2 proliferative response (modified standard curve). Upper right panel: validation of the specificity of the Nb2 bioassay for PRL in CM from preadipocytes. Nb2 cells were co-incubated with CM, collected from IBMX-stimulated preadipocytes (200 μM for 24 h), and anti-PRL antibodies (Ab; 6 μg/well) or rabbit IgG (6 μg/well). Dashed line represents the lower limit of assay sensitivity. Lower panel: stimulation of PRL release in preadipocytes by cAMP activators. Preadipocytes were incubated alone (Con) or with 200 μM IBMX, 1 μM isoproterenol (iso), or 10 nM PACAP. After 24 or 48 h, CM were collected and analyzed for PRL by the Nb2 bioassay. PRL levels in CM containing IBMX were calculated from the modified standard curve. Each value is a mean ± SEM of triplicate determinations. Asterisk (*) designates significant differences from controls ($P < 0.05$). This is a representative experiment repeated at least three times.

pathways, either in parallel or downstream of the elevated cAMP, may be operative. To begin identifying the signaling pathways involved, preadipocytes were incubated with each cAMP activator in the presence or absence of the PKA, PI3K or MEK inhibitors, H89, LY294002, and UO126, respectively. To account for possible direct effects of these inhibitors on Nb2 cells, standard curves were generated in their presence or absence. As shown in Fig. 4, inset, only LY has a significant suppressive effect on Nb2 cell proliferation. Therefore, PRL levels in CM containing IBMX or LY were calculated from the corresponding modified standard curves. Neither protein kinase inhibitor has a significant effect on PRL release from non-stimulated preadipocytes (Fig. 4). All the inhibitors abolished the stimulatory effect of IBMX and significantly reduced PRL stimulation by isoproterenol. Because of the combined suppressive effects of IBMX and LY on Nb2 cell proliferation, the PRL values calculated from the modified curves were below non-stimulated controls. The PKA inhibitor was ineffective against PRL stimulation by PACAP, whereas the PI3K and MAPK inhibitors caused partial and complete inhibition, respectively. Notably, the dissimilarity in basal and ligand-stimulated PRL secretion shown in Figs. 3 and 4, likely reflect variability in the preadipocyte pools from different patients.

Transcriptional activation of the superdistal PRL promoter in primary preadipocytes

To define which elements within the superdistal PRL promoter are active under basal conditions, preadipocytes were transiently transfected with a luciferase reporter driven by the full-length (−3000/+66) decidual PRL (dPRL) promoter or with the deletion mutants shown in Fig. 5, upper panel. Transfection with either the full-length promoter or the −317 construct resulted in a 25-fold increase in luciferase activity above vector control (Fig. 5, lower panel). On the other hand, the −1556 and −675 constructs caused only 5- to 8-fold increases over the control, indicating the presence of inhibitory elements between the putative proximal promoter region and the distal enhancer.

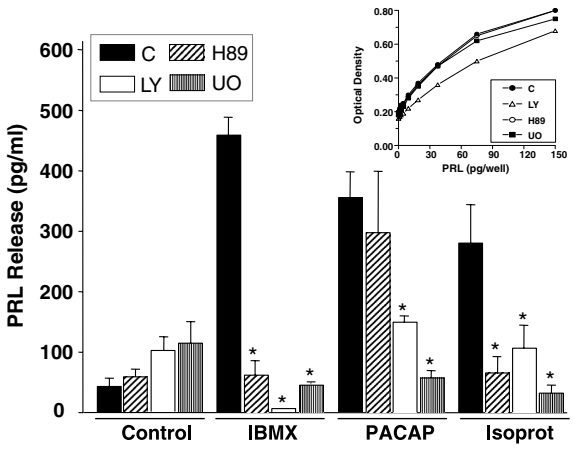

Fig. 4. Effect of protein kinase inhibitors on PRL release from non-stimulated (control) or stimulated preadipocytes, as determined by the Nb2 bioassay. The inset shows that LY294002 (LY, a PI3K inhibitor), but not H89 (PKA inhibitor) or UO126 (UO, a MAPK inhibitor), caused a significant ($P < 0.05$) suppressive effect on the proliferative response of Nb2 cells. Preadipocytes were first incubated with 10 μM of H89, LY or UO for 1 h and then 200 μM IBMX, 1 μM isoproterenol (isoprot), or 10 nM PACAP were added. After 24 h, CM were collected and analyzed for PRL by the Nb2 bioassay. PRL levels in CM containing either IBMX or LY were calculated from the corresponding modified standard curves. Each value is a mean ± SEM of triplicate determinations. Asterisk (*) designates significant differences from controls ($P < 0.05$). This experiment was repeated with similar results.

Discussion

This is the first report on the release of biologically active 23 kDa PRL from human breast preadipocytes and its transient expression during early adipogenesis. PRL gene expression and release were stimulated in preadipocytes by cAMP activators such as isoproterenol and PACAP via PKA and non-PKA signaling pathways. These data also established that PRL transcription in human adipocytes is driven by the superdistal PRL promoter which contains stimulatory and inhibitory domains.

Because the RIA for PRL has insufficient sensitivity, we took advantage of the superior sensitivity of the Nb2 bioassay. To rule out the possibility that Nb2 cells proliferate in response to secretory products of adipocytes other than PRL, we verified assay specificity by PRL immunoneutralization. Additionally, whenever exogenous stimulators/inhibitors were found to exert a direct effect of the Nb2 proliferative response, modified standard curves were used. Metabolic labeling confirmed de novo synthesis of 23 kDa PRL by breast preadipocytes.

PRL expression was transiently increased on days 1 and 3 of differentiation whereas the PRL-R was variably expressed throughout adipogenesis. It is unclear whether PRL-R expression reflects the changing levels of local PRL, which can up- or down-regulate its receptors under

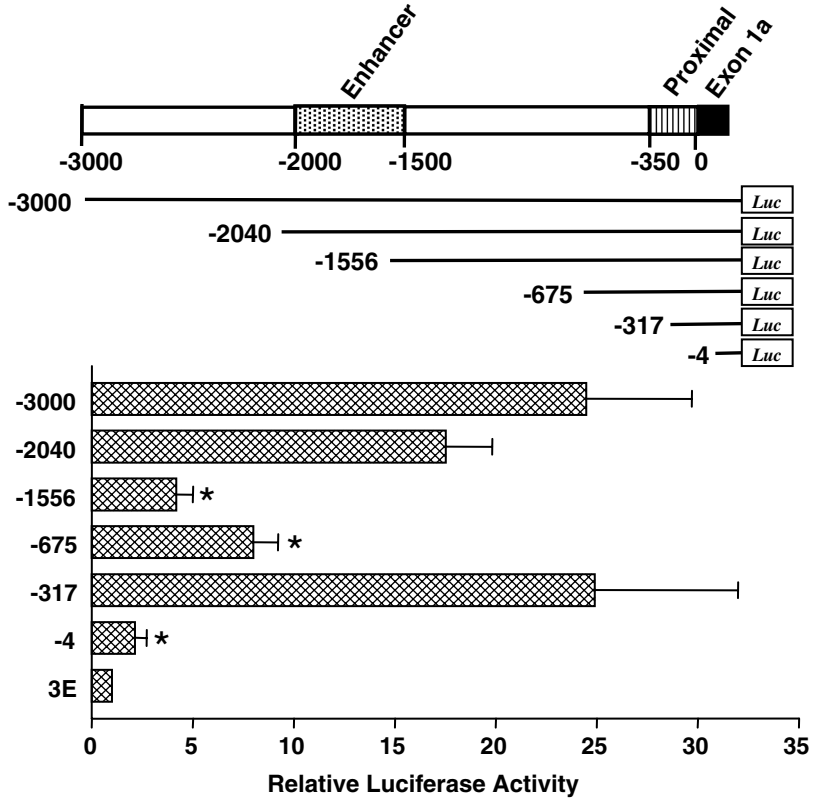

Fig. 5. Upper panel: schematic presentation of the superdistal PRL promoter as deduced from work done with human decidual cells (modified from Brar et al. [30]). The putative proximal promoter region and enhancer domain are shown. Lower panel: relative luciferase activity in preadipocytes transfected with the promoter constructs depicted above. Transfection efficiency, corrected by using Gaussia luciferase, was expressed as fold changes over the PGL3E plasmid, which was assigned a value of 1. Each value is a mean ± SE of triplicate determinations. Asterisk (*) designates significant differences from values of the −3000 full promoter sequence ($P < 0.05$). This experiment was repeated with similar results.

some conditions [16,17]. For example, the PRL-R is increased during adipogenesis of murine BMS2 bone marrow stromal cells [18], but is decreased in LS14 human adipocytes [19]. In addition, expression of STAT 5a/b, the PRL-R downstream transcription factors, is increased during 3T3-L1 murine adipocytes differentiation [20].

Of all the components of the differentiation medium, only IBMX increased PRL gene expression. IBMX was removed after three days because prolonged exposure is toxic to the cells. Although IBMX is required for preadipocyte conversion in vitro [21], the endogenous cAMP activating ligands that are necessary for in vivo adipogenesis are unknown. Hence, it is unclear whether PRL expression is also altered during in vivo adipogenesis. Isoproterenol, epinephrine, PACAP and VIP, all of which bind to G-protein-coupled receptors and increase adenylate cyclase activity, stimulated PRL gene expression and release in preadipocytes. α- and β-Adrenergic receptors are abundant in adipose tissue and mediate catecholamine-induced inhibition and stimulation of lipolysis, respectively [22]. Human adipocytes express the PAC1 receptor [23], and PACAP was recently reported to induce lipolysis in rat adipocytes [24]. In addition to catecholamines and PACAP/VIP, adipose PRL could be regulated by other factors, which may or may not act by increasing adenylate cyclase activity.

Increased intracellular cAMP levels activate the catalytic subunit of PKA. Activated PKA phosphorylates the transcription factor CREB which increases transcription of many target genes. The superdistal PRL promoter is induced by cAMP activators [25], and this is mediated by a CREB complex that binds to a palindromic CRE recognition site (TGACGTCA) at position −12 in the superdistal promoter [26]. In support of our data on the effect of several protein kinase inhibitors, a recent study reported that multiple, PKA-dependent and PKA-independent signals are involved in cAMP-induced PRL expression in a human eosinophilic cell line [27].

The decidual-type superdistal PRL promoter is utilized only in humans and primates [25]. Active elements within this promoter have been mapped in decidual, lymphocytes, and myometrial cells [6,7,25,28,29]. Our studies revealed the presence of two positive regulatory domains which correspond to the proximal region and distal enhancer in decidual cells [30], whereas the sequences between −317 and −1556 likely represent regulation by inhibitory factors. One possible inhibitor is annexin-1, which suppresses PRL in decidual tissue by inhibiting the cAMP pathway at a point after cAMP activation [31]. Other potential inhibitors are several interleukins, including IL-1β, IL-2, and IL-4, which inhibit PRL production in decidual and myometrial cells as well as in lymphocytes [32–34].

A question which was not addressed in this study is whether PRL is supportive or obligatory for the process of adipogenesis in human preadipocytes. This issue can be best addressed using a PRL-producing cell line rather than working with primary cells. Indeed, we recently developed a novel adipocyte cell line, named LS14, from a patient with metastatic liposarcoma [19]. These cells exhibit many properties of primary preadipocytes, including the ability to undergo terminal differentiation and expression of key adipocyte-specific genes. Similar to primary adipocytes, LS14 cells produce and respond to PRL, making them a valuable model to study adipose PRL. PRL production, both at the mRNA and protein levels, increases markedly during early differentiation of LS14 cells. Ongoing studies are developing an siRNA approach to suppress local PRL production and examine its functions during adipogenesis.

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