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Prostacyclin as a potent effector of adipose-cell differentiation

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The terminal differentiation of Ob1771 pre-adipose cells induced by arachidonic acid in serum-free hormone-supplemented medium containing insulin, transferrin, growth hormone, tri-iodothyronine and fetuin (5F medium) was strongly diminished in the presence of inhibitors of prostaglandin synthesis, namely aspirin or indomethacin. Carbaprostacyclin, a stable analogue of prostacyclin (prostaglandin I_2) known to be synthesized by pre-adipocytes and adipocytes, behaved as an efficient activator of cyclic AMP production and was able, when added to 5F medium, to mimic the adipogenic effect of arachidonic acid. Prostaglandins E_2, $F_{2\alpha}$ and D_2, unable to affect the cyclic AMP production, failed to substitute for carbaprostacylin. However, prostaglandin $F_{2\alpha}$, which is another metabolite of arachidonic acid in pre-adipose and adipose cells, able to promote inositol phospholipid breakdown and protein kinase C activation, potentiated the adipogenic effect of carbaprostacyclin. In addition, carbaprostacyclin enhanced both a limited proliferation and terminal differentiation of adipose precursor cells isolated from rodent and human adipose tissues maintained in primary culture. These results demonstrate the critical role of prostacyclin and prostaglandin $F_{2\alpha}$ on adipose conversion *in vitro* and suggest a paracrine/autocrine role of both prostanoids in the development of adipose tissue *in vivo*.

INTRODUCTION

The process of adipose conversion has been delineated *in vitro* with the use mainly of rodent pre-adipocytes from established cell lines [1]. Early events, such as the emergence of lipoprotein lipase, are triggered by growth arrest [2,3], whereas expression of late phenotypes, such as glycerol-3-phosphate dehydrogenase (GPDH) and triacylglycerol accumulation, requires a limited proliferation of these cells expressing early markers of differentiation. This situation, in agreement with data obtained *in vivo* [4,5], strongly suggests that dormant precursor cells already committed can generate new mature fat-cells upon stimulation by specific mitogens. In order to test this hypothesis and to gain a better understanding of the factors involved in the control of this process, serum-free media have been defined [6–9]. We have recently shown, using Ob1771 mouse pre-adipocyte cells maintained in a serum-free hormone-supplemented medium [5F medium containing insulin, transferrin, growth hormone (GH), tri-iodothyronine (T_3) and fetuin], that post-confluent mitoses and terminal adipose differentiation were strictly controlled by cyclic AMP and modulated by messengers generated through hydrolysis of inositol phospholipids [10]. Furthermore, arachidonic acid appeared to be one of the non-protein 'adipogenic' factors present in serum [10]. Arachidonic acid, as a promoter of both the cyclic AMP and the inositol phospholipid pathways, was indeed able to trigger terminal differentiation when added to 5F medium [10]. We have thus investigated the possible involvement of some arachidonic acid metabolite(s) as physiological effector(s) of this process. In the present paper we report that carbaprostacyclin ($cPGI_2$), a stable analogue of prostacyclin (PGI_2) [11,12] known to be synthesized by pre-adipocytes and adipocytes [13–17], is an efficient activator of cyclic AMP production and a potent effector of adipose conversion for both pre-adipocytes of established cell lines and adipose precursor cells isolated from rodent and human adipose tissues cultured in serum-free hormone-supplemented media. In addition prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which is able to activate the inositol phospholipid breakdown pathway, potentiates the adipogenic effect of $cPGI_2$.

EXPERIMENTAL

Methods

Stock cultures of Ob1771 cells (a subclone of Ob17) [18,19] and 3T3-F442A cells [20] were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum as previously described [10,18–20]. After plating in serum-supplemented medium at a density of 8.5×10^3 cells/cm^2 in 24-multiwell Linbro tissue-culture plates (2 cm^2/well), cells were extensively washed 20–24 h later with a mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium (1:1, v/v) and subsequently grown to confluence (3 days) in the same mixture as above supplemented with insulin (5 μg/ml), transferrin (10 μg/ml), a partially purified kallikrein-like activity from rat submaxillary gland (1 μg/ml) [21] and fibroblast growth factor (25 ng/ml) [6]. Confluent cells were then shifted to another serum-free medium consisting of Dulbecco's modified Eagle's medium and Ham's F12 medium mixed as above and enriched with sodium ascorbate (100 μM) and sodium selenite (20 nM) and containing insulin (5 μg/ml), transferrin (10 μg/ml), GH (2 nM), T_3 (200 pM) and fetuin (500 μg/ml) [7,10]. This medium referred to as 5F medium was further supplemented or not as indicated in

Abbreviations used: GPDH, *sn*-glycerol-3-phosphate dehydrogenase (EC 1.1.1.8); GH, growth hormone (somatotropin); T_3, tri-iodothyronine; PG, prostaglandin; $cPGI_2$, carbaprostacyclin; PGI_2, prostacyclin; IBMX, 3-isobutyl-1-methylxanthine; PMA, phorbol 12-myristate 13-acetate.

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the text or in the legends to Figures. Unless otherwise stated, these media were changed at days 3 and 7 after confluence.

Adipose precursor cells present in the stromal–vascular fraction of rodent as well as human adipose tissue (plastic surgical samples) were obtained by collagenase digestion as previously described [8,9]. Cells were plated in serum-supplemented medium at an initial density of 2×10^4 cells/cm^2 (rodent cells) or 10^5 cells/cm^2 (human cells). After attachment and careful washings, cells were fed with a standard medium referred to as ITT medium [8,9] and containing insulin (5 μg/ml), transferrin (10 μg/ml) and T_3 (200 pM) supplemented or not as indicated in the legend to Fig. 3. GPDH activity, protein content, cell count, intracellular cyclic AMP content and inositol phospholipid breakdown were determined as previously described [10]. The final concentration of ethanol, used as a vehicle for all compounds tested in this study, never exceeded 1% and had no effect on either adipose conversion or short-term cellular responses such as the intracellular cyclic AMP content or that of inositol phosphates.

Materials

All prostaglandins were purchased from Cayman Chemicals (Ann Arbor, MI, U.S.A.). Arachidonic acid, indomethacin, aspirin and 3-isobutyl-1-methylxanthine (IBMX) were products of Sigma Chemical Co. (St. Louis, MO, U.S.A.). The sources of other products have already been given [10].

RESULTS

Effect of aspirin and indomethacin on adipose conversion induced by arachidonic acid in Ob1771 cells

We first examined the effect of the non-steroidal anti-inflammatory drugs, aspirin and indomethacin, on the terminal differentiation of Ob1771 pre-adipocyte cells maintained in 5F medium supplemented with 10 μM-arachidonic acid. These drugs are potent inhibitors of the cyclo-oxygenase that catalyses the conversion of arachidonic acid into prostaglandins [13,22]. As shown in Table 1, a 12-day exposure of confluent cells to arachidonic acid in the presence of aspirin or indomethacin led to a strong decrease in the GPDH activity, taken as an enzyme marker of adipose terminal differentiation [1,5,10]. Exposure of the cells for 5 h to 10 μM-indomethacin before arachidonic acid resulted in a more potent inhibition, although shorter times of exposure might also have been effective, according to the data obtained in serum-supplemented medium for prostaglandin synthesis [13]. The decrease observed in the GPDH activity was accompanied by a parallel diminution in the number of differentiated triacylglycerol-containing cells. Indeed, under our serum-free culture conditions, a fairly good correlation was observed between the number of differentiated triacylglycerol-containing cells, easily detected under the microscope, and the specific activity of GPDH measured at day 12: a value of 3000 munits/mg of protein or more was the result of one cell doubling within 6 days after confluence, followed by differentiation and leading in a reproducible manner to 98–100% differentiated cells present as a monolayer [10]. The effect of cyclo-oxygenase inhibitors on adipose-cell differentiation induced by arachidonic acid suggested that the suppression of prostanoid synthesis in Ob1771 cells ($PGE_2 > PGI_2 > PGF_{2\alpha}$ [13]) should also prevent the initial rise in the cyclic AMP content that proved to be essential for terminal differentiation in serum-free hormone-supplemented medium [10]. It was thus not surprising that indomethacin-treated cells, which became unable to differentiate, did not produce cyclic AMP in response to a 5 min exposure to 10 μM-arachidonic acid (Fig. 1).

Table 1. Inhibition by aspirin and indomethacin of adipose conversion induced by arachidonic acid

Confluent Ob1771 cells were maintained during 12 days in 5F medium enriched with 10 μM-arachidonic acid with or without supplementation with aspirin or indomethacin as indicated. Indomethacin was added either simultaneously with (*) or 5 h before (†) arachidonic acid. At day 12 GPDH activity and protein content of cell homogenates were determined as described in the Experimental section. Results are presented as percentages of the specific activity obtained for cells maintained in 5F medium plus arachidonic acid. Values are the means for two determinations that did not differ by more than 8% with cells from two separate culture wells in one series of cells and the medians ± ranges of three independent experiments performed with indomethacin. Neither aspirin nor indomethacin significantly affected the GPDH specific activity of control cells maintained in 5F medium (i.e. in the absence of arachidonic acid), the mean value of which was 30 ± 10 munits/mg.

Drug added	GPDH specific activity (%)
None	100
Aspirin (10 μM)	28
Aspirin (100 μM)	14
Indomethacin (10 μM)*	28 ± 12
Indomethacin (10 μM)†	4.5

Effect of prostaglandins on cyclic AMP production in Ob1771 cells

In an attempt to determine the nature of the arachidonic acid metabolite(s) directly involved in the production of cyclic AMP, we examined the effect of various prostanoids on this parameter. Since PGI_2 is known to decompose rapidly into a chemically stable but biologically inactive derivative, 6-oxo-$PGF_{1\alpha}$, we took advantage of the availability of a stable analogue of PGI_2, namely $cPGI_2$, reported to be active in the prevention of platelet aggregation [11,12]. As shown in Fig. 1, $cPGI_2$ was a potent activator of cyclic AMP production in Ob1771 cells and, as expected, addition of indomethacin did not prevent this effect. Within 5 min 100 nM-$cPGI_2$ was able to increase the intracellular cyclic AMP content to a value similar to that induced by 10 μM-arachidonic acid. In contrast, addition of 200 nM-PGD_2, -PGE_2 or -$PGF_{2\alpha}$ failed to provoke any change in the cyclic AMP content as compared with non-exposed cells. This effect of $cPGI_2$ was in agreement with data showing that prostacyclin stimulates cyclic AMP production in several cell types, including fibroblasts [23–25].

Induction of terminal differentiation and post-confluent mitoses by carbaprostacyclin

When added to 5F medium, $cPGI_2$ was also able within 12 days to trigger, in a concentration-dependent

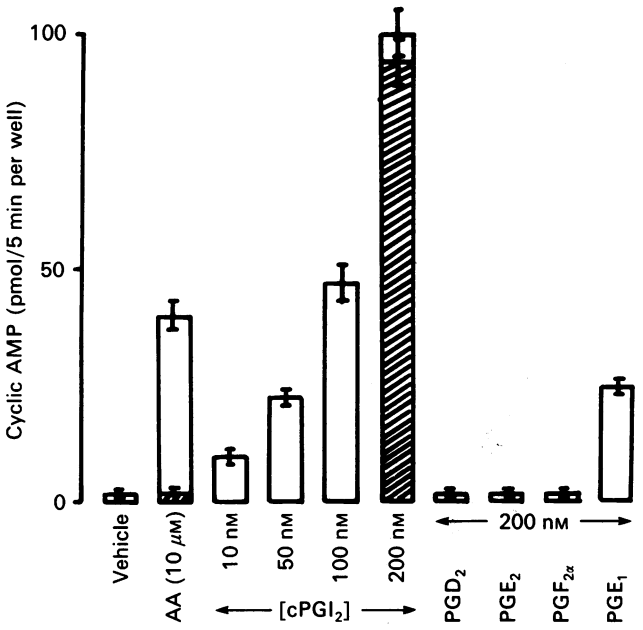

Fig. 1. Effect of arachidonic acid and various prostaglandins on the concentration of intracellular cyclic AMP in confluent Ob1771 cells

Cells grown to confluence were washed and preincubated in 5F medium containing 100 μM-IBMX. After 5 min the medium was aspirated and the cells were incubated in the same fresh medium as above supplemented as indicated under each column either in the absence (white columns) or in the presence (hatched columns) of 10 μM-indomethacin. After 5 min the medium was again aspirated, and cyclic AMP was extracted and assayed at least in triplicate as described in the Experimental section. Values are the means $\pm$ S.E.M. (bars) for 12–24 determinations in at least three independent experiments. Indomethacin did not affect the basal concentration of cyclic AMP. Abbreviation: AA, arachidonic acid.

manner, the terminal differentiation of Ob1771 cells, as illustrated in Table 2 by the expression of high GPDH activity. The dose–response relationship was similar to that obtained for cyclic AMP production (Fig. 1). The maximal effect, obtained at 200 nM, led in a very reproducible manner to the differentiation of at least 80% of the cells. As expected, this effect remained unimpaired in the presence of aspirin or indomethacin (Table 2); in other words, $cPGI_2$ was able to reverse fully the inhibitory effect of aspirin or indomethacin.

After a 6-day exposure to a maximally effective concentration of $cPGI_2$, the increase in cell number reached values close to 2-fold (Fig. 2). This situation was in good agreement with our previous results, which showed that one doubling in the cell number within 6 days led to terminal differentiation of all of the cells [10]. It may be noted that results similar to those presented in Table 2 and Fig. 2 were obtained upon exposure of the cells to 200 nM-$cPGI_2$ for shorter periods (48–72 h) provided that there was the simultaneous presence of 100 μM-IBMX as a cyclic AMP phosphodiesterase inhibitor in order to sustain high concentrations of cyclic AMP. Among the various prostanoids assayed 6-oxo-$PGF_{1\alpha}$, PGD_2, PGE_2 (results not shown) and $PGF_{2\alpha}$ (Table 2 and ref. [10]) were ineffective, at a concentration of 200 nM, in triggering the differentiation of a significant number of cells, whereas PGE_1 and 6-oxo-PGE_1 promoted the differentiation of a low proportion of cells (15–20%); with the last two prostanoids this ability was probably related to the enhancement of cyclic AMP production (see Fig. 1 for PGE_1). PGI_2 thus appeared as the specific metabolite of arachidonic acid involved, through enhancement of cyclic AMP, in the control of terminal differentiation of Ob1771 cells.

Besides its capacity to increase cyclic AMP production rapidly, arachidonic acid was also found to induce rapidly but moderately inositol phospholipid breakdown in Ob1771 cells [10]. Furthermore, we have provided evidence that activation of protein kinase C as well as Ca^{2+} mobilization, provoked by second messengers (diacylglycerol and inositol trisphosphate respectively) generated through hydrolysis of inositol phospholipids [26], could act in concert with agents elevating cyclic AMP concentrations to amplify adipose conversion. Studies in the presence of $cPGI_2$ were therefore also undertaken.

Potentiation of $cPGI_2$-induced adipose conversion by $PGF_{2\alpha}$

It should be recalled that the small but significant release of soluble inositol phosphates induced by 10 μM-arachidonic acid from [^{3}H]inositol-prelabelled Ob1771 cells was entirely prevented by 10 μM-indomethacin [10]. This observation suggested that at least one metabolite of arachidonic acid was involved in the control of inositol phospholipid hydrolysis. $PGF_{2\alpha}$, which is known to be synthesized and secreted by pre-adipocytes as well as adipocytes [13–17], has already been shown to be a potent agonist of inositol phospholipid hydrolysis in Ob1771 cells (EC_{50} 30 nM, [10]). In contrast, PGE_2 (the major metabolite of arachidonic acid in Ob1771 cells [13]) and PGD_2 were inactive. PGI_2 could also be excluded as an activator of this pathway since, when added at concentrations up to 1 μM, $cPGI_2$ was unable to provoke in eight separate experiments any release of labelled inositol phosphates. $PGF_{2\alpha}$ thus appeared as the prostanoid responsible for the release of inositol phosphates induced by arachidonic acid.

Table 2 indicates that addition of $PGF_{2\alpha}$ at a concentration that was maximally active upon inositol phospholipid breakdown (200 nM) did not induce significant adipose conversion of Ob1771 cells. However, in the presence of submaximal concentrations of $cPGI_2$ potentiation by $PGF_{2\alpha}$ was clearly apparent. Note that this positive modulation could actually be reversed when the cells were exposed to high concentrations (200 nM each) of $cPGI_2$ and $PGF_{2\alpha}$. Table 2 further shows that addition of the protein kinase C activator phorbol 12-myristate 13-acetate (PMA), but not that of the Ca^{2+} ionophore ionomycin, was able to potentiate the terminal differentiation of Ob1771 cells. These results support the proposal that a potent rise in cyclic AMP production and protein kinase C activation are acting in concert to modulate this process.

Promotion of adipose conversion of other pre-adipose cells by $cPGI_2$

The promotion of terminal differentiation by $cPGI_2$ was not confined to Ob1771 cells, as we have obtained similar results with 3T3-F442A pre-adipocyte cells [20] maintained under serum-free culture conditions (R. Négrel, D. Gaillard & G. Ailhaud, unpublished work). Furthermore the growth- and differentiation-promoting

Table 2. Induction of terminal differentiation by $cPGI_2$: synergism between $cPGI_2$ and $PGF_{2\alpha}$ or PMA

Confluent Ob1771 cells were maintained for 12 days in 5F medium supplemented or not with the indicated concentrations of $cPGI_2$. Some cultures were also supplemented with either aspirin or indomethacin, $PGF_{2\alpha}$, PMA or ionomycin as indicated. Specific activities of GPDH were determined as described in the Experimental section. Values are reported as medians ± ranges for independent experiments performed with 16 different series of cells (*200 nM-$cPGI_2$) or three different series of cells (all other conditions).

Culture conditions / Addition ...	GPDH specific activity (munits/mg)			
	None	$PGF_{2\alpha}$ (200 nM)	PMA (10 nM)	Ionomycin (100 nM)
5F medium	50 ± 15	150 ± 50	180 ± 55	70 ± 20
+ $cPGI_2$ (10 nM)	80 ± 15	900 ± 80	1050 ± 150	80 ± 20
+ $cPGI_2$ (50 nM)	110 ± 20	1250 ± 110	2700 ± 200	250 ± 100
+ $cPGI_2$ (100 nM)	500 ± 100	–	–	–
+ $cPGI_2$ (150 nM)	1200 ± 110	–	–	–
+ $cPGI_2$ (200 nM)	2800 ± 400*	1500 ± 120	3120 ± 220	2800 ± 400
+ $cPGI_2$ (400 nM)	2900 ± 400	–	–	–
+ $cPGI_2$ (200 nM) + aspirin (100 μM) or indomethacin (10 μM)	2800 ± 300	–	–	–

effects elicited by $cPGI_2$ were not restricted to pre-adipocyte clonal lines. They could be extended to primary cultures of adipose precursor cells isolated from mouse, rat and human adipose tissues (Fig. 3). We have reported [8] that most, if not all, cells of the stromal–vascular fraction of adipose tissue from 4-week-old rats could differentiate into adipocyte-like cells when cultured for 6–10 days in the presence of insulin-like growth factor I and/or insulin, T_3 and transferrin (ITT medium; see Fig. 3*a*). With regard to human adipose tissue, this medium was also able to support, within 2 weeks, the development of small clusters of adipocyte-like cells, although the proportion of differentiated cells remained low (Fig. 3*b* and ref. [9]). Most likely, as already reported for mouse adipose tissue, the stromal–vascular cells should have expressed *in vivo*, i.e. before inoculation *in vitro*, early genes of the differentiation programme such as pOb24 mRNA and the mRNA encoding for lipoprotein lipase [27]. In a way similar to that observed with pre-adipocyte established cell lines, the expression of late markers of differentiation was accompanied in stromal–vascular cells by their limited proliferation [8]. Supplementation of ITT medium with 200 nM-$cPGI_2$ allowed rat precursor cells to accomplish within 6 days one additional round of cell division, which led to a 2-fold increase in the number of differentiated cells (Figs. 3*a* and 3*c*). When comparing Figs. 3(*b*) (ITT medium) and 3(*d*) (ITT medium plus 200 nM-$cPGI_2$), the size of differentiated clusters of human cells was clearly increased when $cPGI_2$ was present. This limited proliferation induced by $cPGI_2$ was also accompanied, in both rodent and human adipose precursor cells, by a 2-fold increase in the specific activity of GPDH (see the legend to Fig. 3).

DISCUSSION

To our knowledge, the above results show for the first time the involvement of PGI_2, in the form of its stable analogue, in a process of differentiation. Our results show that $cPGI_2$ behaves as a factor able to trigger growth and differentiation of early marker-containing pre-adipose cells. Both effects are likely to be triggered through the ability of $cPGI_2$, after binding to a specific receptor, to activate adenylate cyclase, since directly increasing the cyclic AMP content by pharmacological means led to limited proliferation and terminal differentiation [10]. It is reasonable to postulate that PGI_2, which is actually synthesized by Ob17 [13] and 3T3 pre-adipocytes [14,15] as well as by human adipose precursor cells [16], is the mediator of these effects. This mediation probably takes place at low concentrations of PGI_2, since this prostanoid has been reported to be more potent by one order of magnitude than the stable analogue $cPGI_2$ as a platelet anti-aggregating agent [12]. The involvement of labile cellular diffusible factor(s) has been suggested to explain the fact that expansion of differentiating adipose-cell colonies in media containing bovine serum proceeds via mitoses that occur from the centre, where lipid accumulation starts, to the periphery [28]. Our results support the proposal that PGI_2 might play a critical role in that respect, since under our culture conditions addition of the stable analogue led to terminal differentiation of the whole cell population after one cell doubling.

$PGF_{2\alpha}$, which is also synthesized by pre-adipocytes [13–16], has the ability to activate the inositol phospholipid pathway [10] and to potentiate the effect of $cPGI_2$ in promoting adipose conversion. Thus our results support also the proposal that a potent increase in cyclic AMP production and the activation of protein kinase C are acting in concert to modulate terminal differentiation. The absence of potentiation of the $cPGI_2$-induced differentiation by simultaneous exposure to ionomycin (Table 2), in contrast with that observed with other agents elevating cyclic AMP concentrations [10], would suggest that $cPGI_2$, in addition to its effect on cyclic AMP production, might also be able to elicit directly or indirectly a rise in cytoplasmic Ca^{2+} concentration in a manner independent of inositol phospholipid hydrolysis. This hypothesis is supported by the facts that (i) Ca^{2+} influx is regulated by cyclic AMP in several cell types [29] and (ii) PGE_1, which is able to bind to the same cell-surface receptor as prostacyclin [30] induces a rise in

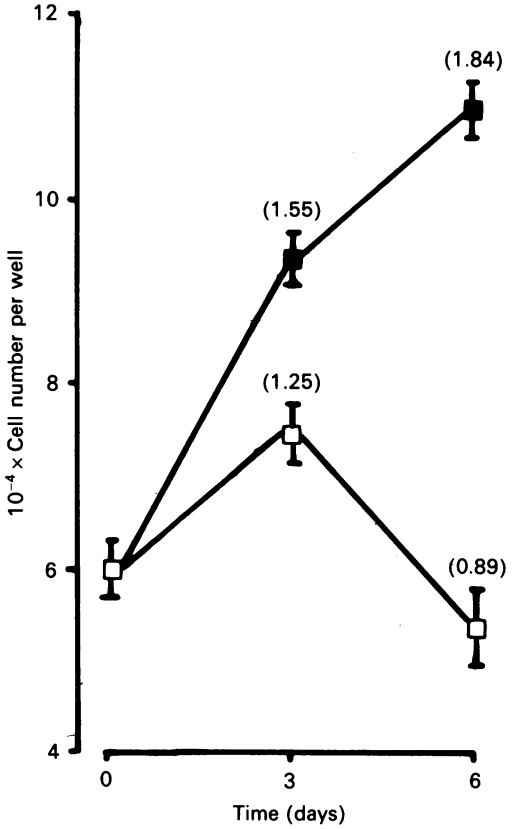

Fig. 2. Induction of post-confluent mitoses by $cPGI_2$

Culture wells maintained in 5F medium supplemented (■) or not (□) with 200 nM-$cPGI_2$ were used for cell counting after treatment with trypsin at day 3 or 6 post-confluence with a Coulter counter. Values are the means ± S.E.M. (bars) for triplicate determinations in three independent experiments. Numbers in parentheses correspond to the mean values of the cell number increase.

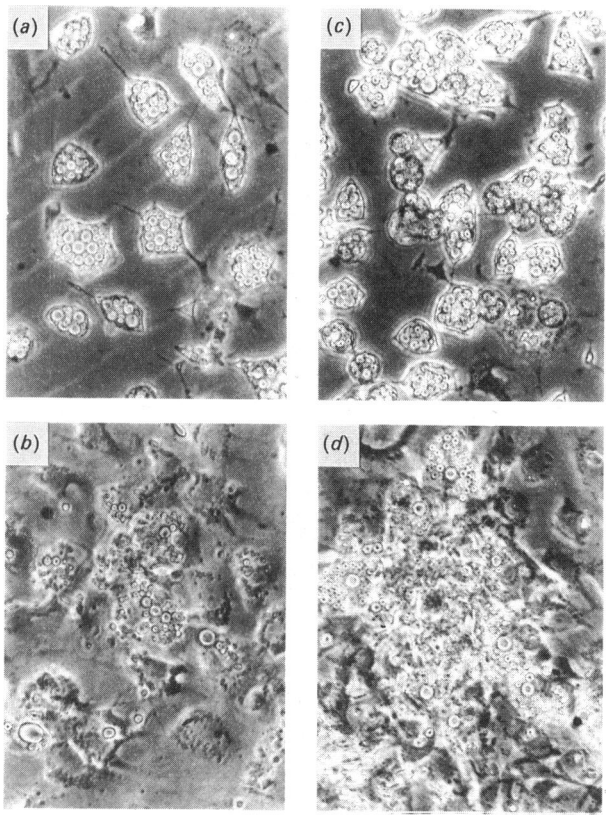

Fig. 3. Promoting effect of $cPGI_2$ on proliferation and differentiation of adipose precursor cells from rat and human adipose tissue in primary culture

Adipose precursor cells present in the stromal–vascular fraction of periepididymal adipose tissue of 4-week-old Wistar rats (*a* and *c*) and of abdominal subcutaneous adipose tissue of a 41-year-old woman (*b* and *d*) were obtained and plated as described in the Experimental section. After attachment and careful washing, cells were fed with ITT medium [8,9] containing insulin (5 μg/ml), transferrin (10 μg/ml) and T_3 (200 pM) in the absence (*a* and *b*) or in the presence (*c* and *d*) of 200 nM-$cPGI_2$. These media were renewed once at day 3 for rat cells and three times at days 3, 7 and 10 for human cells. Microphotographs (magnification × 150) show representative fields of cells maintained under each set of conditions at day 6 (*a* and *c*) and day 14 (*b* and *d*). Accumulation of refringent intracellular triacylglycerol droplets in differentiated cells is clearly visible. Cells were then washed and scraped from the dishes, and cell homogenates were prepared and used to measure GPDH specific activity. Mean values obtained from two separate dishes are 1515 (*a*) and 2930 munits/mg (*c*) at day 6 for rat cells and 28 (*b*) and 54 munits/mg (*d*) at day 14 for human cells. Similar results to those presented in (*a*) and (*c*) have been also obtained with cells isolated from mouse periepididymal tissue as well as rat and mouse subcutaneous adipose tissues. Similar results to those presented in (*b*) and (*d*) have also been obtained with stromal–vascular cells isolated from a lipoma of a 36-year-old woman.

both cyclic AMP concentrations and Ca^{2+} influx in 3T3 cells [31]. Additional experiments should allow confirmation of this assumption.

As the result of a combined action of PGI_2 and $PGF_{2\alpha}$ (Fig. 4), the activation of specific protein kinases accounts for most, if not all, of the promoting effect of arachidonic acid on the process of adipose conversion [10]. It is proposed that the model given in Fig. 4 applies to the situation *in vitro*, in which the prostaglandins are acting after secretion as autocrine modulators of the mitoses of early marker-containing cells, leading in turn to terminal differentiation. This model probably applies also to the situation of adipose-tissue development *in vivo* in which prostaglandins could be secreted by dormant adipose precursor cells and/or surrounding cells, among which are capillary endothelial cells and mature adipocytes, known to synthesize and secrete PGI_2 and $PGF_{2\alpha}$ [17,32]. According to this model, besides PGI_2 and $PGF_{2\alpha}$ production, the prior mobilization of arachidonic acid, through a phospholipase A_2-(PLA_2-) rather than a phospholipase C- (PLC-)dependent mechanism should play a central role. This feature takes into account the fact that the amount of diacylglycerol (DAG), generated upon stimulation of the hydrolysis of polyphosphoinositides (PIP_2) by a maximally effective concentration of $PGF_{2\alpha}$ in Ob1771 cells, did not seem to be an efficient source of arachidonic acid, since no differentiation took place under these conditions (Table 2). Therefore binding of undefined physiological effectors to their cognate receptors (R_X) may activate phospholipase

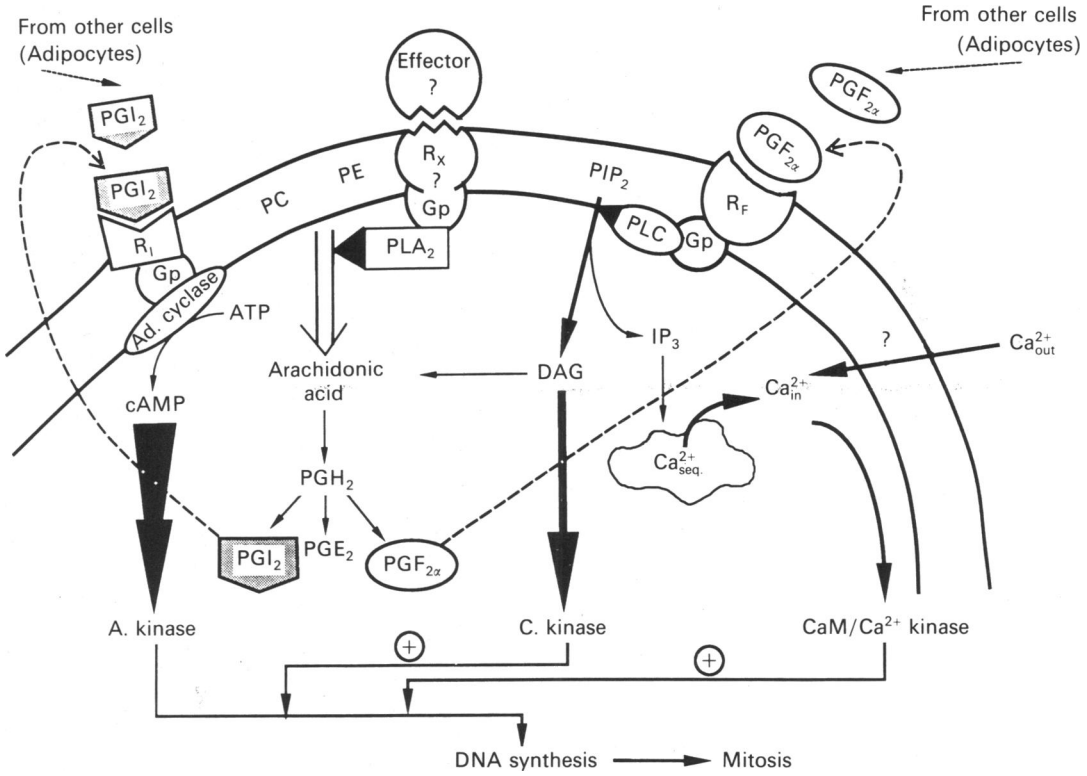

Fig. 4. Putative autocrine/paracrine role of PGI_2 and $PGF_{2\alpha}$ in the control of adipose terminal differentiation *in vitro* and of adipose tissue development *in vivo*

PGI_2 and $PGF_{2\alpha}$ synthesized from arachidonic acid, after exit from the cell, interact with their specific cell-surface receptors R_I and R_F. Binding to these receptors, coupled via GTP-binding proteins (Gp) to adenylate cyclase (Ad. cyclase) and phospholipase C (PLC) respectively, generates cyclic AMP (cAMP) on the one hand and diacylglycerol (DAG) and inositol trisphosphate (IP_3) from phosphatidylinositol 4,5-bisphosphate (PIP_2) on the other hand. Inositol trisphosphate is known to release sequestered Ca^{2+} ($Ca^{2+}_{seq.}$) from intracellular stores and to increase free Ca^{2+} (Ca^{2+}_{in}). The pool of free Ca^{2+} can also be filled from the extracellular space (Ca^{2+}_{out}) via channel(s) in the plasma membrane (arrow). The various kinases (A. kinase, cyclic AMP-dependent kinase; C. kinase, Ca^{2+}/phospholipid-dependent kinase; CaM/Ca^{2+} kinase, calmodulin + Ca^{2+}-dependent protein kinase) activated by cyclic AMP, diacylglycerol and Ca^{2+} control DNA synthesis and the critical mitosis (or mitoses) leading to GPDH expression and terminal differentiation. A major role is ascribed to the cyclic AMP-dependent phosphorylation pathway, which is permissive for the mitogenic response (large arrow). Modulation exerted by other phosphorylation pathways is indicated by ⊕. The mobilization of arachidonic acid through phospholipase A_2- (PLA_2-)-dependent hydrolysis of phosphatidylcholine (PC) or phosphatidylethanolamine (PE) is hypothetically depicted as involving receptors (R_X) for still unknown effectors coupled to the enzyme via GTP-binding proteins (question marks). PGI_2 and $PGF_{2\alpha}$ locally produced by other cells such as mature adipocytes could play a similar role as described above. No particular role can be ascribed to PGE_2, which is nevertheless the major product of arachidonic acid in both mouse and human adipose precursor cells [18,23,24]. Putative positive or negative controls between each of the various signalling pathways are not presented.

A_2 via a putative GTP-binding protein (Gp). We further postulate that this mechanism represents an important source of free arachidonic acid in these cells.

The nature of the hormonal factors able to trigger arachidonic acid mobilization and prostaglandin synthesis remains an open question, although vasoactive peptides and catecholamines are known to be effectors of these processes in rat as well as in human adipocytes [33–35]. *In vivo*, both dormant adipose precursor cells and mature adipocytes, able to synthesize PGI_2 and $PGF_{2\alpha}$ [13–17], could be the potential targets for these hormonal factors. In any event, once released, these prostanoids could play, in a paracrine/autocrine manner and provided that the appropriate hormonal milieu was present, a critical role in the regulation of terminal differentiation and therefore in the control of adipose-tissue cellularity.

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