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Catecholamine Stimulated Lipolysis in Differentiated Human Preadipocytes in a Serum-Free, Defined Medium

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Abstract Adipocyte precursors from the stromal vascular fraction of human adipose tissue were allowed to differentiate in serum-free defined medium, whereafter their catecholamine stimulated lipolytic response was compared to that of mature isolated human adipocytes. Seventy-five to ninety percent of the fibroblast-like cells accumulated lipid droplets and glycerol-3-phosphate dehydrogenase activities of 1,000–2,800 mU/mg protein were measured in cell homogenates of differentiated cells. Lipolysis could be stimulated by both isoproterenol and norepinephrine in both differentiated preadipocytes as well as mature adipocytes. The results obtained with β-adrenergic agents suggested the presence of a higher affinity receptor in differentiated preadipocytes as compared to mature adipocytes. Mature adipocytes responded well to β-adrenergic agents, but no antilipolytic α_2-adrenergic response was observed in the differentiated preadipocytes. The presence of Gi proteins in the differentiated preadipocytes was suggested by the antilipolytic effect of adenosine as well as the lipolytic activity generated by pertussis toxin. In conclusion, our medium supported the differentiation of a very high percentage of human preadipocytes which developed a sensitive β-adrenergic lipolytic response but which lacked an α_2-adrenergic antilipolytic response.

Key words: adipocytes, β-adrenergic agents, α_2-adrenergic agents, svf

The lipolytic response of adipocytes from various mammalian species has been studied extensively using isolated, mature adipocytes. It is well known that lipolysis is stimulated through the activation of β-adrenergic receptors in all these species [Lafontan et al., 1990], while only some possess antilipolytic α_2-adrenergic receptors. These include the hamster [Carpene et al., 1980], dog [Berlan et al., 1982], rabbit [Lafontan, 1981], and human [Lafontan and Berlan, 1980].

Although isolated adipocytes provide an excellent model to study the short-term effects of various hormones and other agents on lipolysis in vitro, they usually survive for a limited period only after isolation. This makes it difficult to study any long-term effects that these agents might exert on adipocytes.

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As an alternative, various preadipocyte cell lines have been successfully used for investigations of this kind [Lai et al., 1982; Pou and Torresani, 1989; Stadel et al., 1987]. Although clonal cell lines are very convenient to use, they have a few major disadvantages. Firstly, no human preadipocyte cell line has been developed, and it is well known that species differ with respect to their lipolytic response [Lafontan et al., 1990]. Secondly, fetal bovine serum can promote differentiation in some of these cell lines, but it has the opposite effect in human preadipocyte cultures [Hauner et al., 1989]. Thirdly, they are aneuploid and immortal, which could introduce differences between them and normal cells.

Preadipocytes isolated from the stromal vascular fraction (svf) of adipose tissue can also be used to study the development of the lipolytic response in adipocytes. Only one report on the lipolytic response in human preadipocytes could be found [Roncari et al., 1980], but these studies were performed on preadipocytes at a very early stage of differentiation. In differentiated rat pre-

adipocytes, catecholamines only weakly stimulated adenylate cyclase compared to adipocytes [Kirkland et al., 1987], while differentiated hamster preadipocytes responded to β-adrenergic but not to α_2-adrenergic agents [Lafontan et al., 1990], except when cultured for an extended period of time, in which case the latter response started to develop [Saulnier-Blache et al., 1991].

These studies indicate that preadipocytes that differentiate in culture are potentially good models for lipolytic studies, but some work still needs to be done in order to find conditions in which the fully differentiated cell more closely resembles the mature adipocyte with respect to lipolytic responsiveness.

Whereas a large number of cells in the svf of rat [Deslex et al., 1987] and hamster [Lafontan et al., 1990] adipose tissue are able to differentiate into adipocytes in culture, the highest rate of differentiation reported for human preadipocytes to date is 70%, and this can only be obtained in a serum-free medium [Hauner et al., 1989]. In the present study we report on a serum-free, defined medium for human preadipocytes in which up to 90% of the cells differentiated to form multilocular fat cells. Their catecholamine stimulated lipolytic and antilipolytic responses were investigated and compared to those of freshly isolated, mature adipocytes.

MATERIALS AND METHODS

Chemicals

All standard chemicals obtained from commercial sources were of analytical reagent grade and were used without further purification. Collagenase Type II from *Clostridium histolyticum*, Lima bean trypsin inhibitor Type-II-l, (−)-norepinephrine bitartrate, (−)-isoproterenol, DL-propranolol-HCl, clonidine-HCl, Triiodothyronine (T_3), dexamethasone, 3-isobutyl-1-methyl-xanthine (IBMX), and fetuin were obtained from Sigma Chemical Company (St. Louis). RX-821002A was purchased from Research Biochemicals Inc. (Natick, MA). Transferrin and bovine insulin (both tissue culture reagents) and bovine serum albumin, fraction V (BSA), were purchased from Boehringer Mannheim. The BSA was defatted prior to use [Ramachandran et al., 1972]. (−)3-[^{125}I]Iodocyanopindolol ([^{125}I]CYP), approximately 2,000 Ci/mmol, was purchased from Amersham Radiochemical Centre.

Adipose Tissue

With permission from the relevant authorities, abdominal or mammary human adipose tissue was obtained from healthy female subjects undergoing cosmetic surgery. They were all between the ages of 18 and 38 years and had a body mass index varying between 20 and 26 kg/m^2. The tissue was transported to the laboratory in physiological saline at room temperature as soon as possible after removal.

Isolation and Culture of Adipocyte Precursors

A large piece of adipose tissue was immersed in 70% ethanol for 30 sec, after which approximately 60 g was dissected out and freed from any visible connective tissue. Care was taken not to use any of the tissue from the outer layers that were exposed to the ethanol. The tissue was minced well with a blade and washed with sterile saline to remove most of the blood. Collagenase digestion (a modification of the method described by Rodbell [1964]) was carried out at 37°C in a gyratory shaking bath for 90 min in a Krebs-Ringer bicarbonate buffer (1 ml/g tissue) containing 2 mg/ml collagenase. To assist the dispersion of the tissue, the flask was lightly shaken by hand at 15 min intervals. At the end of the incubation, the adipose tissue digest was filtered, first through a 250 μm and then through a 75 μm nylon mesh. The adipose tissue digest was centrifuged for 10 min at 175*g* and the pellet was suspended in a 1:1 (v/v) mixture of Dulbecco's modification of Eagle's minimum essential medium and Ham's F12 (DMEM:F12) containing penicillin and streptomycin (0.01% (w/v) each) and 10% fetal bovine serum. The cells were plated into 10 cm tissue culture dishes at a very high density of 1.6–2.0 × 10^5 cells/cm^2 and left for 18 h, after which time the medium was replaced by the following defined medium: DMEM:F12 containing insulin (10 μg/ml), transferrin (10 μg/ml), T3 (10^{-9} M), and dexamethasone (10^{-8} M). This high plating density proved to be necessary, as it was clear that the seeding efficiency at this stage tended to be low (microscopic observation). The attached cells, being in a subconfluent state, were allowed to grow in this medium, reaching confluence in 3–5 days, at which time they were detached using trypsin/EDTA in phosphate buffered saline (containing no calcium or magnesium). They were then suspended in DMEM:F12 containing 10% fetal bovine serum and plated out into 12-well tissue

culture plates (Corning) at a cell density of 7.6×10^4 cells/cm^2. After 3 h, the subconfluent cells were washed twice with DMEM:F12 and fed the same serum-free, defined medium as before, but also containing fetuin (100 μg/ml), HDL (75 μg protein/ml), and LDL (75 μg protein/ml). Confluence was reached after 2–4 days, when 0.1 mM IBMX was added to the medium. The cells were fed once a week and IBMX was removed after 1 week. All cultures were kept at 37°C in a humidified atmosphere containing 5% CO_2.

Isolation of Mature Adipocytes

Isolated mature adipocytes were obtained by collagenase digestion of human adipose tissue fragments in Krebs-Ringer bicarbonate buffer using a modification [Van Heerden and Oelofsen, 1989] of Rodbell's method [Rodbell, 1964].

Determination of the Degree of Differentiation

Glycerol-3-phosphate dehydrogenase (G3PDH) activity, a late marker of adipocyte differentiation, was used as a measure of the degree of differentiation [Wise and Green, 1979]. Protein was determined using the method of Lowry et al. [1951]. Lipid droplets and cell nuclei were stained with Oil-Red-O and hematoxylin, respectively [Thompson, 1966].

Assays for Lipolytic Activity

The amount of glycerol released into the incubation buffer upon stimulation by lipolytic agents was used as a measure of lipolysis. Cell cultures were normally ready for lipolytic studies at approximately 16–20 days after their isolation from the tissue. The growth medium was then removed from the cells and replaced by Krebs-Ringer bicarbonate buffer containing 4% BSA (0.5 ml/well), which also contained the lipolytic agents. Incubations were carried out for 3 h at 37°C in an atmosphere of 5% CO_2–95% air. Freshly isolated, mature adipocytes were incubated likewise in an atmosphere of 5% CO_2–95% O_2 for 90 min in polypropylene tubes in a total volume of 0.55 ml. At the end of the incubation, 2 aliquots of 200 μl each of the infranatant buffer were removed and used to determine the amount of glycerol released by the cells, according to a modification of the Korn [1955] procedure [Van Heerden and Oelofsen, 1989]. Quantification of DNA was performed as described previously [Van Heerden and Oelofsen, 1989].

Preparation of Crude Adipocyte Membranes From Differentiated Preadipocytes

The following method was adapted from Lai et al. [1981]. All steps were performed on ice or at 4°C. Human preadipocytes were allowed to differentiate in 10 cm culture dishes using the method described above. The cell layers were washed twice in buffer A (10 mM potassium phosphate, pH 7.4, containing 0.14 M NaCl). They were then scraped from the plate in buffer B (5 mM Tris-HCl, pH 7.4, containing 5 mM NaCl, 0.1 mM EGTA, and 1 mM dithiothreitol) using a teflon scraper. The cells were left in this buffer for 3 min, after which time $MgCl_2$ was added to a final Mg^{2+} concentration of 0.5 mM. After 2 min, the cells were homogenised, using a Potter-Elvehjem homogeniser with a teflon plunger. The homogenate was centrifuged at 45,000*g* (15 min, 4°C), and the pellet was suspended in buffer A at a concentration of 2–3 mg protein/ml. The crude membrane fraction was immediately stored in aliquots at −60°C and used for receptor binding assays within 2 weeks. Protein was determined using the method of Lowry et al. [1951].

Receptor Binding Assays

Binding assays with [^{125}I]CYP were performed in polypropylene tubes in a total volume of 250 μl, containing 25–30 μg of membrane protein per tube. Incubations were carried out for 60 min at 37°C in a 50 mM Tris-HCl buffer (pH 7.4) containing 10 mM $MgCl_2$ and 0.68 mM ascorbic acid. Non-specific binding was determined as the amount of [^{125}I]CYP that could bind in the presence of 10^{-5} M propranolol. At the end of the incubation the tubes were placed on ice and immediately filtered through Whatman glass-fibre filters under low vacuum on a filter manifold. The filters were rapidly washed three times with 3.3 ml cold 0.9% NaCl solution and used for determination of radioactivity.

Isolation of HDL and LDL

Human HDL and LDL were isolated by sequential ultracentrifugation from the plasma of normal male blood according to the methods described by Strachan et al. [1988] and Srinivasan et al. [1989], respectively.

Calculations and Data Analysis

Non-linear regression. Lipolysis data were analysed using the BMDPAR derivative-free non-

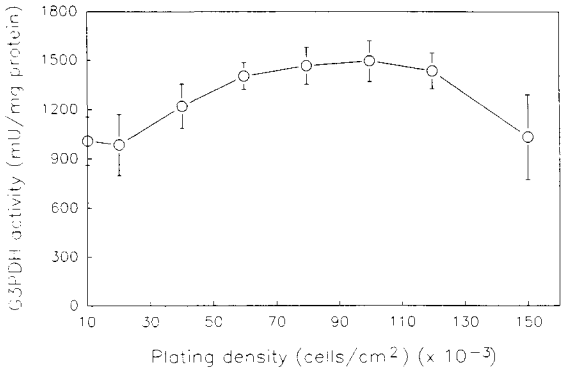

Fig. 1. The effect of plating density after trypsinisation on the differentiation of human preadipocytes in serum-free medium. Cells were cultured as described in Materials and Methods. The data points shown represent the mean (±SEM) for two experiments.

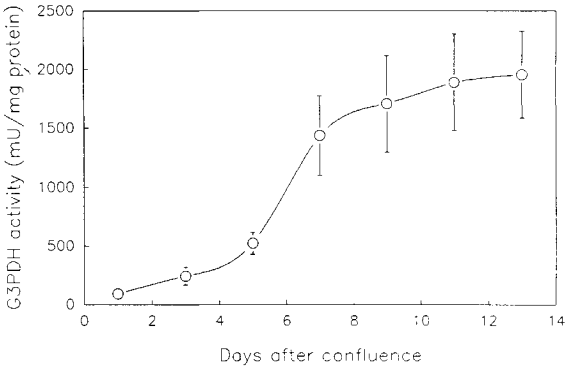

Fig. 2. Time study of the appearance of glycerol-3-phosphate dehydrogenase activity in human preadipocytes in culture. Cells were cultured in serum-free medium as described in Materials and Methods. The data points shown represent the mean (±SEM) for three experiments. Where no error bars are shown, the SEM values lie within the symbols.

linear regression program developed by the department of Biomathematics, UCLA, as revised for PC version May 1984 (copyright 1983, Regents of University of California).

The equation used for lipolysis response curves was that for a rectangular hyperbola. All results reported were corrected for basal lipolysis. Results were compared using the Student's t-test.

RESULTS

Differentiation of Human Preadipocytes in Serum-Free Culture

After the collagenase digestion, many of the cells were found in clusters, which made it difficult to get an even cell suspension. It was also observed that most of the cells that ultimately differentiated were in these clusters. This same observation was made by Deslex et al. [1986]. As a result of this, the number of differentiating cells was not always the same in different wells. The introduction of the trypsinisation step after the cells had grown for 3–5 days brought about a great improvement in this regard. The cells had a fibroblast-like appearance and reached confluence 2–4 days after trypsinisation and replating. Differentiation was dependent on the plating density after trypsinisation, and it was found that a density of 6–12 × 10^4 cells/cm^2 gave optimum results (Fig. 1). A small portion of the preadipocytes started to accumulate fat droplets at about 2–3 days post confluence, while the activity of the enzyme G3PDH started to increase rapidly approximately 2 days later (Fig. 2). This activity leveled off 10–12 days after confluence, when 75–90% of the cells had differentiated into mature, multilocular fat cells (Fig. 3). The cells never acquired a unilocular appearance, because they tended to tear loose from the substrate due to their buoyancy. Addition of defatted BSA to a final concentration of 4% at this point delayed the floating of the cells only for a day or two. BSA was therefore not added to the cultures during the present study. Maximum values for G3PDH activities obtained in different cultures varied between approximately 1,000 and 2,800 mU/mg protein. The G3PDH activities of cultured preadipocytes were compared to those obtained from human adipose tissue extracts after removal of visible connective tissue (Table I). The values for adipose tissue ranged between 700 and 2,000 mU/mg, and the average value was not significantly different from that of the differentiated preadipocytes.

Comparison of the Lipolytic Responses of Cultured and Mature Adipocytes

Both norepinephrine and isoproterenol stimulated lipolysis in differentiated preadipocytes that were kept in serum-free culture for 16–20 days (Fig. 4A). As could be expected, isoproterenol, an agonist with a higher affinity for β-adrenergic receptors than norepinephrine, has a lower ED_{50} (the ligand concentration at which a half-maximal response was obtained) than norepinephrine (11 ± 9.3 nM [SEM; n = 8]) as compared to 248 ± 169 nM (SEM; n = 5), respectively (Table II). Likewise, in the mature adipocytes the average ED_{50} value for isoproterenol was lower than that for norepinephrine (164 ± 35 nM (SEM;

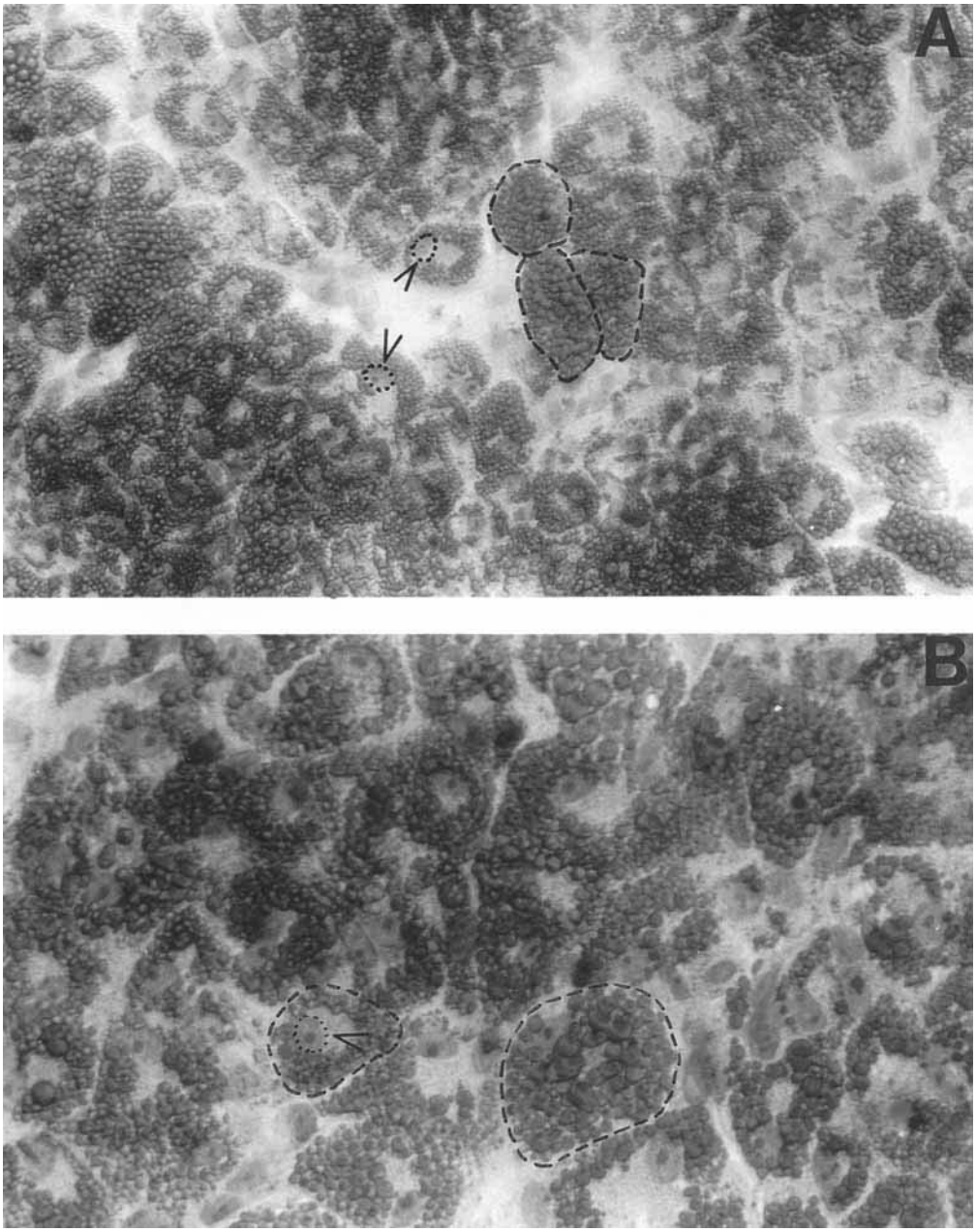

Fig. 3. Differentiated human preadipocytes grown in serum-free culture (12 days post confluence). Culture conditions are described in Materials and Methods. Lipid droplets were stained with Oil-Red-O and appeared as black spots. Individual cells are outlined with pecked lines and arrowheads point to cell nuclei. **A:** ×100. **B:** ×200.

n = 23) and 1,354 ± 276 nM (SEM; n = 22), respectively (Table II). For mature adipocytes, this difference was statistically significant ($P < 0.001$). For differentiated preadipocytes, on the other hand, the difference was not significant, possibly because the values for n were much smaller than in the case of mature adipocytes. Furthermore, the ED_{50} values for isoproterenol were significantly lower ($P < 0.05$) in cultured cells as compared to cells that differentiated in vivo. This could indicate that the β-adrenergic receptors of the differentiated preadipocytes were of a higher affinity than those of the mature adipocytes. A similar trend was observed with regard to the ED_{50} values for norepinephrine action (Fig. 4; Table II). From Figure 4, it appeared as if this difference was large, but it should be noted that Figure 4 represents

TABLE I. Comparison of the G3PDH Activities From Undifferentiated and Differentiated Preadipocytes and Adipose Tissue Extracts†

Source	n	G3PDH activity (mU/mg protein)
Preadipocytes at confluence	6	34.3 ± 5.6
Differentiated preadipocytes	6	*1,605 ± 235
Crude adipose tissue extract[a]	6	*1,100 ± 171

†Values are means ± S.E.M. of n determinations. Adipose tissue was freed of all visible connective tissue before homogenisation.
[a]Unpublished data, TC Koekemoer.
*Significantly higher than the value for preadipocytes at confluence ($P < 0.001$).

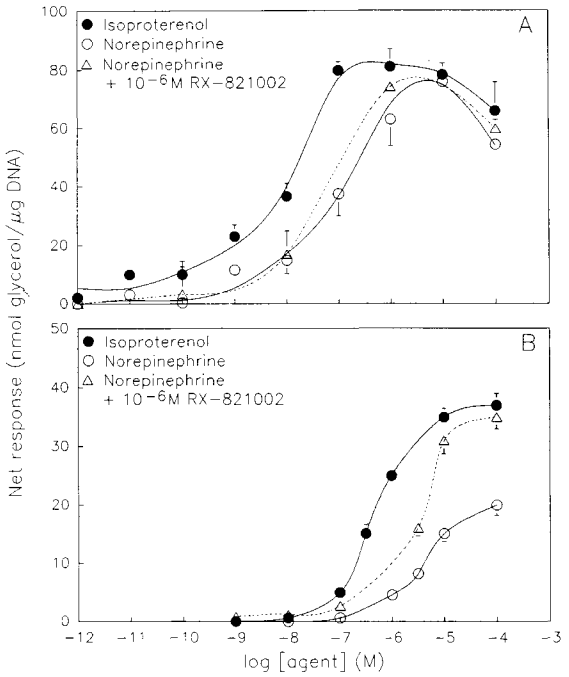

Fig. 4. Log-dose response curves of isoproterenol and norepinephrine stimulated lipolysis in differentiated human preadipocytes (**A**) and mature adipocytes (**B**), and the effect of 10^{-6} M RX-821002 on NE stimulated lipolysis. Incubations were carried out as described in Materials and Methods. Each point represents the mean (±SEM) of duplicate determinations. Data shown are from a single experiment. Each experiment was repeated several times, yielding similar results (variability in ED_{50} and V_m is indicated in Table II). Where no error bars are shown, the SEM values lie within the symbols.

results from single experiments. When all the available data were combined (Table II), the difference was not statistically significant. Despite the difference in affinities between cultured and mature adipocytes towards isoproterenol, their lipolytic capacities (V_m) in response to

TABLE II. Maximum Lipolytic Responses (V_m) and Half-Maximal Effective Concentrations (ED_{50}) for Isoproterenol and Norepinephrine Stimulated Lipolysis in Differentiated Human Preadipocytes in Culture as Compared to Mature Human Adipocytes†

	n	ED_{50} (nM)	V_m (nmol glycerol/μg DNA)
Differentiated preadipocytes			
Isoproterenol	8	*11 ± 9.3	**53 ± 11.6
Norepinephrine	5	248 ± 169	**50 ± 18.0
Mature adipocytes			
Isoproterenol	23	***164 ± 35	***57 ± 5.7
Norepinephrine	22	1,354 ± 276	26 ± 3.9

†Values are means ± S.E.M. of n determinations.
*Significantly lower than the corresponding value for mature adipocytes ($P < 0.05$).
**Significantly different from the corresponding value for norepinephrine stimulated lipolysis in mature adipocytes ($P < 0.05$).
***As in **, except $P < 0.001$.

this agonist were quite comparable (Table II). However, the V_m towards norepinephrine was significantly lower ($P < 0.05$) in mature adipocytes (26 ± 3.9 nmol glycerol/μg DNA) as compared to cultured adipocytes (50 ± 18 nmol glycerol/μg DNA). Furthermore, in isolated mature adipocytes, the maximum lipolytic response obtained with norepinephrine was also significantly lower than that for isoproterenol ($P < 0.001$) (Fig. 4B; Table II). This can be explained by the previously reported presence of antilipolytic α_2-adrenergic receptors on adipocyte cell membranes [Lafontan and Berlan, 1980]. This antilipolytic response was observed irrespective of whether adenosine deaminase was present in the system or not. On the other hand, the maximum responses obtained with these two adrenergic agonists in cultured preadipocytes were very similar (Fig. 4A; Table II), suggesting the possible lack of development of α_2-adrenergic receptors under the culture conditions used in the present study. This was supported by the observations that the α_2-adrenergic antagonist RX-821002 (10^{-6} M) had virtually no effect on the maximum response obtained with norepinephrine in preadipocytes, whereas it increased the norepinephrine stimulated response in mature adipocytes to the same maximum as that obtained with isoproterenol (Fig. 4; Table III). Addition of 10^{-4} M clonidine (α_2 agonist) together with 10^{-6} M isoproterenol

TABLE III. Effect of α_2-Adrenergic Agents on Isoproterenol and Norepinephrine Stimulated Lipolysis in Differentiated Preadipocytes and Mature Adipocytes*

	Lipolytic response (%)	
Adrenergic agents	Mature adipocytes	Differentiated preadipocytes
Isoproterenol (10^{-6} M)	100	100
Norepinephrine (10^{-5} M)	47 ± 3.7	98 ± 1.7
Isoproterenol (10^{-6} M) + Clonidine (10^{-4} M)	54 ± 4.3	99 ± 2.5
Norepinephrine (10^{-5} M) + RX-821002 (10^{-6} M)	98 ± 2.4	99 ± 2.9

*Results are the means (±SEM) for three experiments, each performed in duplicate.

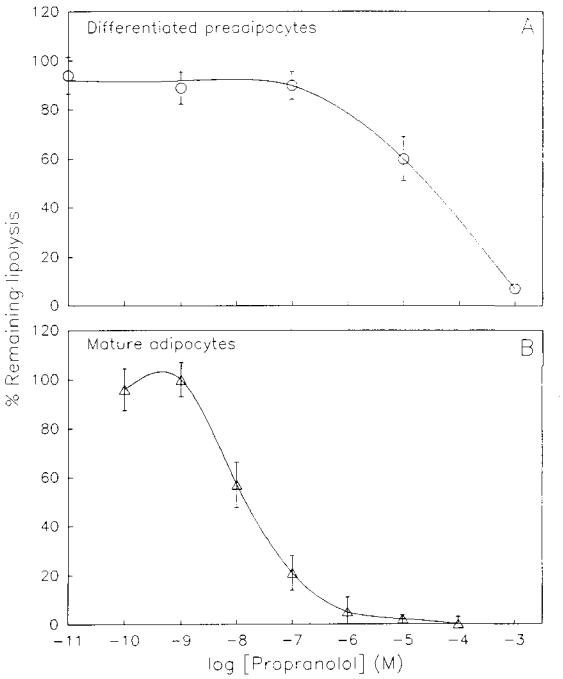

Fig. 5. Inhibition of isoproterenol (10^{-6} M) stimulated lipolysis by propranolol in differentiated human preadipocytes (**A**) and mature adipocytes (**B**). Each point represents the mean (±SEM) of three experiments, each performed in duplicate.

also indicated the absence of functional α_2-adrenergic receptors on differentiated human preadipocytes. The α_2 agonist was not able to suppress isoproterenol stimulated lipolysis in the latter cells, as opposed to freshly isolated, mature adipocytes, where 10^{-4} M clonidine inhibited the lipolytic response by 54 ± 4.3% (SEM; n = 3) (Table III).

The effect of the β-adrenergic antagonist, propranolol, on isoproterenol stimulated lipolysis in the two types of adipocytes was also investigated. Complete inhibition of isoproterenol stimulated lipolysis in differentiated preadipocytes could only be achieved at a relatively high concentration (10^{-3} M) of propranolol (Fig. 5A), whereas only 10^{-6} M propranolol was sufficient for complete inhibition of lipolysis in mature adipocytes (Fig. 5B). The mean ED_{50} values from three experiments on mature adipocytes and three experiments on differentiated preadipocytes were 8.1 ± 0.1 nM (SEM) and 56.2 ± 6.5 μM (SEM), respectively. The reason for this large difference in sensitivity to propranolol is not clear. Although it could be partly due to higher receptor affinities in the cultured cells, as was suggested by the lipolysis results (Table II), this may not be the only reason. As the aim of this particular experiment was to confirm that the lipolytic response in the cultured preadipocyte was mediated via the well-known β-adrenergic pathway, it was decided to further identify the receptors by means of binding studies using the selective β-antagonist [^{125}I]CYP.

Binding of [^{125}I]CYP to Human Preadipocyte Membranes

The presence of β-adrenergic receptors in differentiated preadipocytes was confirmed with binding studies using [^{125}I]CYP as the ligand. Saturation binding studies with crude membrane preparations from differentiated human preadipocytes revealed that the receptors were of high affinity and that the specific binding was saturable (Fig. 6). Scatchard analysis of the results yielded a single, straight line with the mean values for Kd (binding affinity) being 136.7 ± 16.1 pM (SEM) and for the binding capacity, 27.6 ± 11.2 fmol/mg protein (SEM) for three experiments.

Investigation of the α_2-Antilipolytic Response

The apparent absence of an α_2-antilipolytic response observed in the cultured preadipocytes represented an important deviation from mature adipocytes that have differentiated in vivo. Notwithstanding the observation that α_2-adrenergic agents, RX-821002 and clonidine, had no effect on the lipolytic response in cultured adipo-

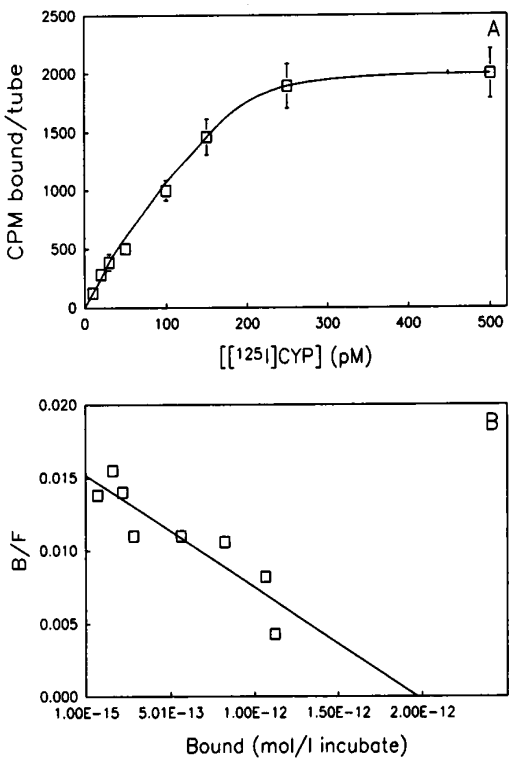

Fig. 6. Saturation binding of [^{125}I]CYP to membranes (31 μg protein/tube) from human preadipocytes that had differentiated in serum-free culture (**A**) and Scatchard analysis of these results (**B**). Each data point represents the mean (±SEM in A) of duplicate determinations (corrected for nonspecific binding) from a single experiment. The experiment was repeated twice, yielding similar results. The mean values for Kd and binding capacity from these experiments are reported in the text. Where no error bars are shown in A, the SEM values lie within the symbols.

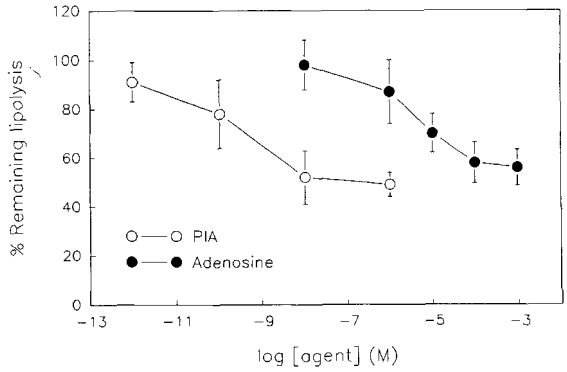

Fig. 7. Inhibition of isoproterenol (10^{-6} M) stimulated lipolysis by PIA and adenosine in human preadipocytes differentiated in culture. Each data point represents the mean (±SEM) of two experiments, each performed in duplicate.

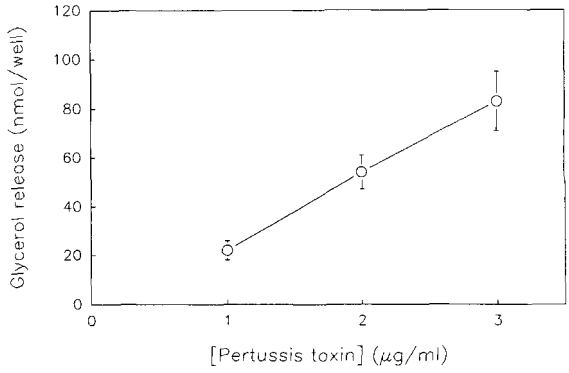

Fig. 8. Stimulation of lipolysis by pertussis toxin in human preadipocytes differentiated in culture. Each data point represents the mean (±SEM) of duplicate determinations from three experiments.

cytes (Table III), it was nevertheless possible to inhibit the isoproterenol stimulated lipolytic response with adenosine and its analogue PIA (Fig. 7). This suggested that Gi proteins were present in these cells and that the lack of an α_2-antilipolytic response was probably due to the absence of functional α_2-adrenergic receptors. The presence of Gi proteins was further verified when it was found that pertussis toxin was a very potent lipolytic agent in the cultured human preadipocytes (Fig. 8).

DISCUSSION

Preadipocytes have been isolated from the stromal vascular fraction of adipose tissue of various species, and in most cases very high rates of differentiation were observed [Lafontan et al., 1990; Deslex et al., 1987]. The highest rate of differentiation reported to date for human preadipocytes, however, was 70% [Hauner et al., 1989]. We have developed a serum-free, defined medium in which up to 90% of the fibroblast-like cells from the stromal vascular fractions of human adipose tissue differentiated. These cells accumulated visible lipid droplets (Fig. 3) and exhibited a large increase in the activity of the adipocyte marker enzyme G3PDH (Fig. 2), which ranged from approximately 1,000–2,800 mU/mg cell protein. This variation could be donor age related, but more studies are needed in order to establish this. The ages of our subjects ranged from 18–38 years. Hauner et al. [1989] reported a decrease in the maximum G3PDH activity observed with an increase in age, but from their results it appeared as if the activity only started to decrease in cells from subjects over 40 years old. It therefore seems unlikely that the variation in our results could be related to age differences alone. Past history of weight loss and gain or parity may be factors in the variation observed.

Not much is known about the lipolytic response of preadipocytes that have differentiated in culture. Kirkland et al. [1987] have shown that the adenylate cyclase activity of rat preadipocytes grown in a serum-containing medium was much lower than that of mature adipocytes. Furthermore, rat preadipocytes seem to have a poorly developed Gi-dependent pathway [Lu et al., 1988]. This may explain why adenylate cyclase was stimulated by adenosine in the latter cells, whereas adenosine usually has an inhibitory effect on adenylate cyclase in isolated adipocytes [Lu et al., 1988]. Saulnier-Blache et al. [1991] have cultured hamster preadipocytes that responded to β-adrenergic agonists 8 days after confluence and to α_2-adrenergic agonists between 20 and 30 days after confluence.

In the present study, a comparison between the lipolytic responsiveness of differentiated preadipocytes (12 days post confluence and total culture period approximately 20 days) and freshly isolated, mature adipocytes revealed the following (Fig. 4; Table II): the lipolytic capacities of the two systems towards isoproterenol were similar; the affinity towards isoproterenol was significantly higher ($P < 0.05$) in cultured preadipocytes than in mature adipocytes, as can be seen from the differences in ED_{50} values; the maximum lipolytic response of mature adipocytes towards NE was significantly lower ($P < 0.001$) than the isoproterenol stimulated response in the same cells, as well as the NE stimulated response in differentiated preadipocytes ($P < 0.05$); and there was no significant difference between the norepinephrine and isoproterenol responses in differentiated preadipocytes. The latter finding suggested the lack of an α_2-antilipolytic response in the cultured cells. The presence of β-adrenergic receptors on differentiated preadipocytes was confirmed by inhibiting the isoproterenol stimulated lipolytic response with propranolol (Fig. 5). Direct evidence for the presence of β-adrenergic receptors in differentiated preadipocyte membranes was obtained from receptor binding studies with [^{125}I]CYP. Scatchard analysis of saturation binding data revealed the presence of a single class of high affinity sites (Kd = 136.7 ± 16.1 pM [SEM; n = 3]) (Fig. 6).

The decrease in ED_{50} for the lipolytic response towards isoproterenol in cultured adipocytes as compared to mature adipocytes could be caused by one (or more) of the additions to the defined culture medium. Steroids [Lai et al., 1982; Xu et al., 1990] as well as T_3 [Pou and Torresani, 1989] have been shown to influence the lipolytic response in various types of preadipocytes in culture. Preliminary results in our laboratory have shown that at least dexamethasone, IBMX, and T_3 can alter the lipolytic response of human preadipocytes when the exposure times or the concentrations are altered. It may therefore be possible to obtain a lipolytic response in the differentiated preadipocytes that compare more closely to that of mature adipocytes by adjusting certain components of the medium.

A more important deviation from mature adipocytes was the apparent absence of an α_2-adrenergic antilipolytic response. This response is believed to play an important role in the overall regulation of human adipose lipolysis in vivo, and only a few other species express these receptors in their adipocytes [Berlan et al., 1982; Carpene et al., 1980; Lafontan, 1981; Lafontan and Berlan, 1980]. The absence of an α_2-antilipolytic response has also been observed in hamster preadipocytes in primary culture, but the response did develop after a prolonged culture period (20–30 days) [Saulnier-Blache et al., 1991]. It is furthermore known that adipocytes from young hamsters also lack an antilipolytic response, but it develops as the animal gets older [Carpene et al., 1983]. In the present study, the total culture period was at least 19 and sometimes as many as 24 days, after which time there was still no evidence of an antilipolytic response, although the presence of functional Gi proteins was indicated (Figs. 7, 8). Human preadipocytes therefore appear to behave differently during their development in culture. This possibility is strengthened by the finding that, in contrast to young hamsters, adipocytes from infants are not very responsive towards norepinephrine, because of the presence of a high concentration of α_2-adrenergic receptors which decreases with age [Marcus et al., 1988]. Some other mechanism might be responsible for the apparent lack of an α_2 response in cultured human preadipocytes. Further studies on this aspect are under way at present in the author's laboratory.

In conclusion, our defined serum-free medium supports the differentiation of up to 90% of the preadipocyte cells derived from the stromal vascular fraction of human adipose tissue. These cells develop very high G3PDH activities and are responsive to β- but not α_2-adrenergic agents. The capacity of the developed β-adrenergic lipolytic response compares well with that observed in isolated mature adipocytes, but the

preadipocytes respond with a significantly higher affinity than the isolated adipocytes.

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