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Androgen receptors in cultured rat adipose precursor cells during proliferation and differentiation: regional specificities and regulation by testosterone

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Different studies suggest that sex hormones affect adipose tissue metabolism and deposition. To investigate the possibility that androgens may play a role in adipose tissue development, we have studied androgen receptors (AR) in rat adipose precursor cells from two different anatomical fat deposits, one deep intraabdominal (epididymal) and one subcutaneous (inguinal) during the proliferation and differentiation processes. AR were quantified by [^{3}H]R1881 specific binding in whole cells and the nuclear fraction and were localized by immunocytofluorimetry in both the cytosol and the nucleus. During the proliferative phase, total AR level decreased from D3 to D6. At confluence (D5), AR were higher in epididymal (64 ± 4 fmol/mg protein) than in subcutaneous (33 ± 3 fmoles/mg proteins) preadipocytes and were up-regulated by testosterone but not by 5α-dihydrotestosterone or by 17β-estradiol. At differentiation (D10-11), nuclear AR decreased by 50% in both precursor fat cell populations when compared to the confluent state (D5) and AR were no more up-regulated but rather down-regulated by testosterone. Because AR are present in preadipocytes and are differently regulated by testosterone depending on the stage of proliferation and differentiation, this study suggests that testosterone may play a role in the control of the adipogenic process.

Keywords: Androgen receptors; cultured preadipocytes; regulation; adipogenesis; regional specificities

Introduction

Sex steroids are known to exert complex actions on metabolism and development of adipose tissue (Wade *et al.*, 1985; Pecquery *et al.*, 1988, 1990; Bjorntorp, 1991; Lacasa *et al.*, 1991). Furthermore, regional differences have been found in the metabolic activity of adipose tissue (Krotkiewski *et al.*, 1983; Rebuffe-Scrive *et al.*, 1987; Dieudonne *et al.*, 1992) and sex hormones appear to play an important role in the mechanisms underlying these regional differences (Rebuffe-Scrive *et al.*, 1985, 1989).

However, it is still unclear whether the effects of sex steroid hormones on adipose tissue result from direct or indirect interactions of these hormones with fat cells.

High affinity specific receptors for estrogens have been described in rat (Gray & Wade, 1980; Pedersen *et al.*, 1991) and recently in human adipose tissues (Mizutani *et al.*, 1994) suggesting that rat and human fat cells are target cells for estrogens. The presence of androgen receptors (AR) has been also reported in male rat adipose precursor cells (De Pergola *et al.*, 1990) and in human adipose tissue (Miller *et al.*, 1990). More recently, we have identified AR in both male hamster white adipocytes and their precursor cells and shown that the number of AR was higher in adipocytes and preadipocytes from the intraabdominal than from the subcutaneous regions (Jaubert *et al.*, 1993). We also found that the level of AR was either upregulated in preadipocytes or down-regulated in mature adipocytes by androgens *in vivo*. In addition, mature adipocytes were found to possess less AR than their precursor cells present in the adipose stroma vascular fraction, suggesting reduction of the AR expression during adipogenesis.

These observations have prompted us to compare the characteristics of AR and their regulation by androgens in cultured rat adipose precursor cells from different localizations during the proliferation and differentiation processes. Results of the present study demonstrate that AR levels (measured by whole cell and nuclear assays) in adipose precursor cells depend on their anatomical localization, their stage of proliferation and differentiation and on the presence of testosterone.

Results

AR binding assays were realized using [^{3}H]R1881, an AR agonist chosen because of its high stability to metabolic conversion (Bonne *et al.*, 1975).

Specificity of the [^{3}H]R1881 binding sites towards different steriods was assessed in confluent preadipocytes by studying the displacement of [^{3}H]R1881 by two unlabelled steroids: 5α-dihydrotestosterone (DHT) and 17β-estradiol. As shown in Figure 1, calculation of the half maximal inhibitory concentration (IC50) indicated that estradiol is hundred times less potent than DHT in displacing [^{3}H]R1881 specific binding. Furthermore, the DHT IC50 value found (3.15 nM) is consistent with binding to androgen receptor binding sites in cells other than preadipocytes (Prins, 1987).

Since the sensitivity of adipose tissue to androgens was shown to vary according to the tissue localization (Pecquery *et al.*, 1988, 1990; Lacasa *et al.*, 1991; Dieudonne *et al.*, 1994), we have compared AR levels in confluent epididymal and inguinal subcutaneous precursor cells using both the whole cell and the nuclear binding assays.

In the whole cell assays, Scatchard analysis of [^{3}H]R1881 specific binding at equilibrium revealed a two-fold higher density (B_{max}) of AR in epididymal than in subcutaneous preadipocytes ($P<0.05$). However, the AR affinity towards [^{3}H]R1881 was similar in both cell populations (Table 1).

To evaluate the number of functional AR, we have studied the AR present in the nuclear fraction of adipose precursor cells at confluence. Binding assays were performed with a saturable concentration of [^{3}H]R1881 (10 nM), and here again, the same site-related difference in nuclear AR number was observed, e.g. about two times more AR in the nucleus of epididymal than of subcutaneous preadipocytes. It must be noticed that in the preadipocytes from both fat deposits, the number of nuclear AR averaged one third of the total

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	IC 50 (nM)
17 ß ESTRADIOL	482 ± 62
DIHYDROTESTOSTERONE	3.15 ± 0.7

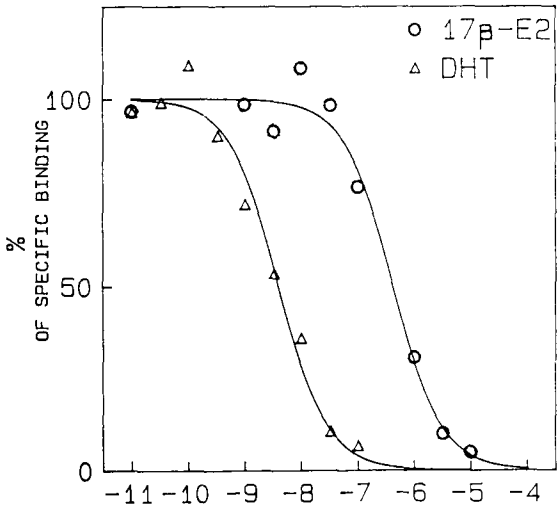

Figure 1 Specificity of [^{3}H]R1881 whole cell binding in confluent (D5) epididymal adipose precursor cells. Precursor cells were incubated in the presence of 10 nM of [^{3}H]R1881 with unlabelled 17β-estradiol and 5α-dihydrotestosterone (DHT) at the indicated concentrations and triamcinolone (10 μM). The inset shows the IC_{50} values for both steroids. Each value represents the mean ± SEM of three separate experiments

AR number found in the whole cell assays (Table 2). This subcellular repartition was confirmed by immunocytofluorimetric studies (Figure 2) showing a specific anti-AR labelling in both the nucleus and the cytosol of confluent preadipocytes.

In a previous study (Jaubert *et al.*, 1993), we have shown that AR level was lower in adipose tissue than in preadipocytes present in the stroma vascular fraction. In order to follow the evolution of AR number during the adipose differentiation process, we have measured the [^{3}H]R1881 specific binding at a saturable concentration of ligand (10 nM) in epididymal and subcutaneous adipose precursor cells at different times of culture (from D3 to D10-11 days culture).

As shown in Figure 3, the AR number assessed by the whole cell binding assay remained rather constant in subcutaneous adipose precursor cells from D3 to D10. In contrast, whole cell AR level decreased in epididymal preadipocytes during the same period. However, in differentiated cells (compared to the undifferentiated confluent state D5), the nuclear AR number was reduced by approximately one half in adipose precursor cells from both fat localizations (Figure 4). Death or alteration of the cells could not account for this decrease in AR number, since at D10, trypan blue was excluded by 100% of the cells and DNA and protein levels were still higher than for confluent cells (not shown). At D10, the G3PDH values were 1633 ± 16 and 1179 ± 127 mU/mg protein in epididymal and subcutaneous cells respectively. In hamster, we have recently reported (Jaubert *et al.*, 1993) that the AR levels are down-regulated in mature adipocytes and conversely, up-regulated in preadipocytes from the same fat deposits by androgen treatment *in vivo*. In rat adipose tissue as well, we observed AR down-regulation by androgens *in vivo* in mature

Table 1 Whole cell [^{3}H]R1881 specific binding in confluent (D5) adipose precursors from epididymal and subcutaneous fat deposits.

	Epididymal	*Subcutaneous*
B_{max} (fmol / mg protein)	64.5±8.5	34±1*
k_d (nM)	1.38±0.6	1.51±0.49

*$P<0.05$. Cells were incubated 1 h at 37°C with various concentrations (0.5-5 nM) of [^{3}H]R1881 in the presence or the absence of dihydrotestosterone (1 μM). B_{max} and K_d values were obtained from Scatchard analysis and represent the mean ± SEM of three separate experiments

Table 2 Whole cell and nuclear [^{3}H]R1881 specific binding in confluent (D5) adipose precursors from epididymal and subcutaneous deposits

	Epididymal	*Subcutaneous*
Intact cell binding (fmol / 10^6 cells)	7.8±0.51	3.6±0.31 ** (−54%)
Nuclear binding (fmol / 10^6 cells)	2.7±0.34	1.15±0.27* (−57%)

*$P<0.05$. **$P<0.0001$. (%), % of epididymal. Cells were incubated for 1 h at 37°C with saturable concentration of [^{3}H]R1881 (10 nM) in the presence or the absence of dihydrotestosterone (1 μM). Whole cell or nuclear binding assay were performed as described under Materials and Methods. The values represent the means ± SEM of four to six separate experiments

adipocytes (sham operated = 10.5 fmoles/mg protein, castrated = 50 fmoles/mg protein, castrated + testosterone = 13.5 fmoles/mg protein) (unpublished results).

These observations led us to compare the direct influence of androgens *in vitro* on primary cultured confluent and differentiated rat preadipocytes obtained from both epididymal and subcutaneous fat deposits.

Confluent (D5) or differentiated (D10) monolayers were incubated for 24 h with 0.1 μM of testosterone. At the end of incubation, both the nuclear and whole cell androgen bindings were assayed. As shown in Figure 5, addition of testosterone during the proliferative phase induced a two to threefold increase in AR number in both whole cells and nuclear fractions whatever the anatomical origin of the preadipocytes. This upregulatory effect appears to be specific to testosterone since 5α-dihydrotestosterone (0.1 μM) and 17β-estradiol (0.1 μM) were without any effect (data not shown). In differentiated cells however, the up-regulatory effect of testosterone could no longer be observed (Figure 5) since whole cell AR were rather down-regulated and nuclear AR unchanged after exposure to testosterone. Again, cell viability, assessed by trypan blue exclusion, was not altered by prolonged exposure to testosterone.

Discussion

It is well established that steroid hormone actions are mediated by specific intracellular receptors in their target cells. In adipose tissue where estrogen receptors have been identified (Gray & Wade, 1980; Pedersen *et al.*, 1991; Mizutani *et al.*, 1994), estrogens have been shown to modulate adipogenesis by increasing the replication of adipose precursor cells (Roncari & Van, 1978). AR have been also characterized in adipose tissue from various species including human and hamster (Miller *et al.*, 1990; Jaubert *et al.*, 1993). More recently, the presence of these receptors has been reported in rat and hamster adipose precursor cells (De Pergola *et al.*, 1990; Jaubert *et al.*, 1993). Since the AR levels per cell found in fully mature adipocytes (i.e. those present in adipose tissue) were low in comparison with their precursor

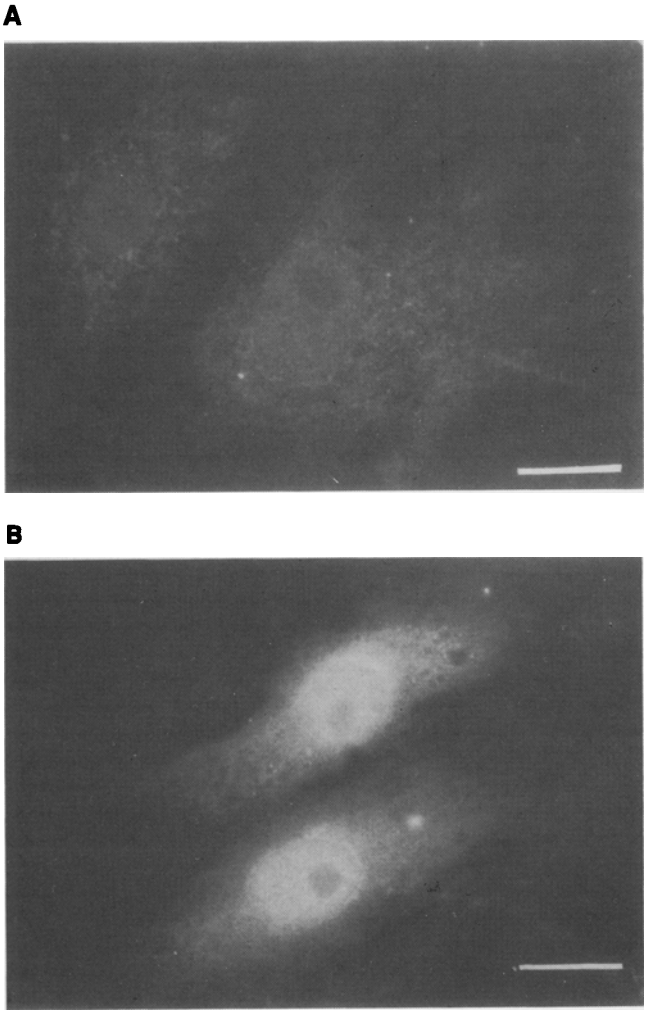

Figure 2 Photomicrographs of AR immunocytofluorimetry in adipose precursors cells. (**A**) Cells were incubated with rabbit IgG for 24 h at + 4°C (negative control). (**B**) Cells were incubated with the specific PA1-110 anti-AR antibody under the same conditions. Scale bar = 15μm

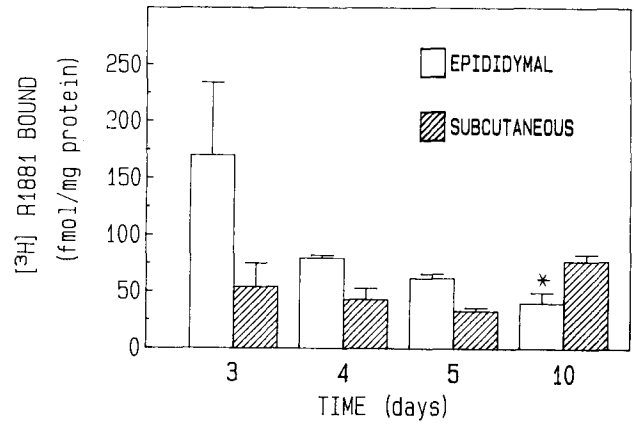

Figure 3 Time course of [^{3}H]R1881 specific binding to intact adipose precursor cells. For each indicated day of culture, cells were incubated 1 h at 37°C in the presence of 10 nM of [^{3}H]R1881 and whole cell binding assays were performed as described under Materials and methods. Each value represents the mean ± SEM of three separate experiments. *$P<0.05$ as compared to D4.

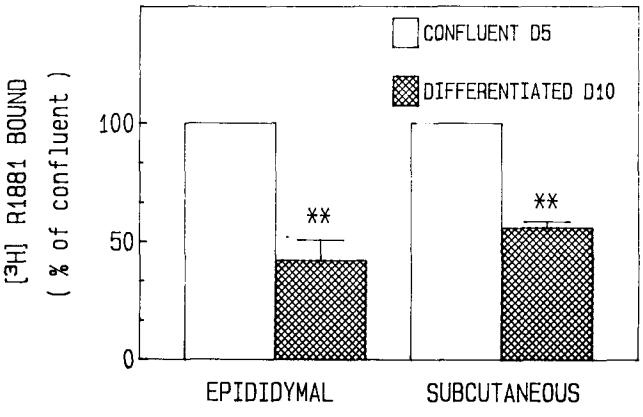

Figure 4 Comparison of [^{3}H]R1881 nuclear specific binding between confluent and differentiated adipose precursor cells. Confluent or differentiated precursor cells were incubated in the presence of 10 nM of [^{3}H]R1881 and nuclear binding assays were performed as described under Materials and Methods. Each value represents the mean ± SEM of three different experiments. **$P<0.005$.

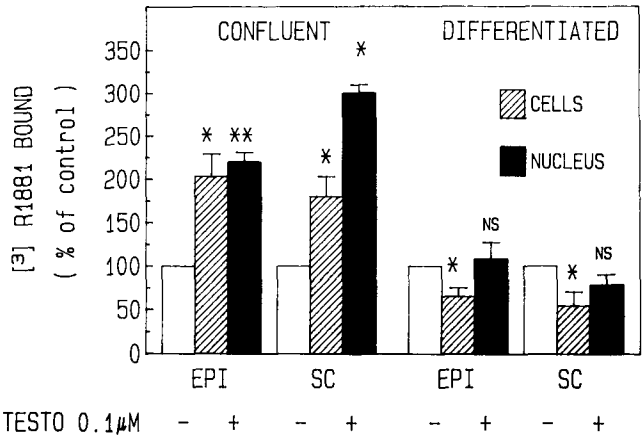

Figure 5 Effect of testosterone on cellular and nuclear [^{3}H]R1881 specific binding in epididymal and subcutaneous precursor cells. Cells were incubated 1 h at 37°C in the presence of 10 nM of [^{3}H]R1881 after exposure for 24 h to 0.1 μM testosterone. Nuclear or whole cell assays were performed as described under Materials and methods. The open bars represent control values obtained without testosterone and normalized to 100%. Each value represents the mean ± SEM of three separate experiments. *$P<0.05$, **$P<0.005$, NS: not significant, EPI: epididymal, SC: subcutaneous

cells (i.e. the preadipocytes of the stroma vascular fraction), the present work was designed to follow the evolution of AR number during adipogenesis. Preadipocytes maintained in primary culture were thus studied during the proliferation and differentiation processes. Moreover, since site-related differences were reported in adipose tissue AR levels (Miller *et al.*, 1990; Jaubert *et al.*, 1993), this study has been conducted with preadipocytes from both the epididymal and the subcutaneous fat deposits. By using the whole cell and nuclear [^{3}H]R1881 binding assays, we demonstrated that AR are more abundant in preadipocytes from epididymal than from subcutaneous adipose tissue. Since the same situation was found in mature adipocytes, it can be concluded that the site-related differences in adipocytes AR already exist in the very early stage of adipogenesis and is maintained throughout the adipogenic process. It seems likely that these site-related differences in AR (Roncari & Van, 1978; Miller *et al.*, 1990) and also ER (Pedersen *et al.*, 1991) equipments may contribute, at least in part, to explain the differences between intraabdominal and subcutaneous fat cells in terms of their sensitivity to androgens (Dieudonne *et al.*, 1994) or estrogens (Lacasa *et al.*, 1991). Indeed, we and others have shown, that androgens regulate, *in vivo* and *in vitro*, the α2 adrenergic receptor expression (Dieudonne *et al.*, 1994; Pecquery *et al.*, 1995) and the adenylate cyclase activity in mature fat cells (Pecquery *et al.*, 1990) as well as in preadipocytes (Xu *et al.*, 1993; Lacasa *et al.*, 1994), subcutaneous adipose tissue being

systematically less sensitive to androgens than deep intraabdominal deposits.

However, the evolution of AR level during *in vitro* adipogenesis was the same in epididymal and subcutaneous precursor cells: nuclear AR number (which represents the functional AR) was half decreased from D3 (confluence) to D10-11 (differentiated stage) of culture.

This reduction was also observed by measuring the total AR number (whole cell assays) in epididymal precursor cells but not in subcutaneous precursor cells. One possible explanation for these discrepancies could be that the AR distribution between the nucleus and the cytosol is not the same in epididymal and in subcutaneous adipose tissue in which AR could mainly fractionate into the cytosolic fraction (Blok *et al.*, 1991).

A decrease in steroid hormone receptors was also observed in cultured cells of different origin (MCF7 cells, hepatocytes, fibroblasts) and it was generally concluded that cytosolic AR or ER are unstable in the absence of hormone in the culture medium (Syms *et al.*, 1985; Berthois *et al.*, 1990; Grino *et al.*, 1990; Freyschuss *et al.*, 1993). In our model, it is hard to say that the decrease in AR number that occurs during *in vitro* adipogenesis is solely related to the absence of testosterone in the culture medium. As a matter of fact, *in vivo*, preadipocytes have more AR than the fully mature adipocytes from the same fat deposits (Jaubert *et al.*, 1993). On the other hand, the present study shows that the addition of testosterone in the culture medium up-regulates its own receptors in adipose precursor cells during the proliferation but not the differentiation phase. Moreover, addition of 5α-dihydrotestosterone or 17β-estradiol had no effect on AR number, indicating that the upregulatory effect of testosterone on AR does not require conversion to 5α-dihydrotestosterone nor to estrogens. However, the question of why this up-regulation was not observed in differentiated cells remains unsettled. It only indicates that a dramatic change occurs in the sensitivity and even in the responsiveness of the cells to androgens during the preadipocyte differentiation process *in vitro*. Occurrence of such a change is further supported by our *in vivo* findings that in rat (present study) and in hamster (Jaubert *et al.*, 1993) testosterone treatment induces a down-regulation of AR in mature adipocytes.

Positive and negative autologous regulation of both AR mRNA and AR protein have been demonstrated in several cellular types (Quarmby *et al.*, 1990; Blok *et al.*, 1991; Krongard *et al.*, 1991; Rossini, 1991) but the mechanism of this dual autoregulation is still unclear. Recent experiments from our laboratory demonstrate that in female rats AR are present in adipose precursor cells at the same level than in male and are also upregulated by testosterone *in vitro* (unpublished observations). Since we have shown that AR were not regulated by 17β-estradiol, it can be postulated that some of the steroid precursors of androgens are probably involved in this AR upregulation. Finally, it cannot be excluded that the differences between confluent and differentiated preadipocytes concerning AR regulation are related to differences in their aromatase activity. Indeed, it was shown that in adipose precursor cells cytochrome P450 aromatase mRNA is more abundant than in mature adipocytes (Price *et al.*, 1993).

In conclusion, this study shows that AR are present in preadipocytes and are sensitive to positive regulation by androgens only during the early phases of adipose tissue growth but not during the differentiation process takes place. We can thus reasonably hypothesize that androgens lead to the transcriptional activation of specific androgen regulated genes only during the proliferative phase and then switch to an inactive period during the differentiation process when AR are less abundant. Thus, besides estrogens which have mitogenic effects in adipose precursor cells (Roncari & Van, 1978), androgens may also contribute, through regulation of their own receptors, to the control of the adipogenic process.

Materials and methods

Animals

Male Sprague-Dawley rats (100–150 g) were kept under controlled lighting conditions (light: 6 am, dark: 8 pm) and constant temperature (21°C). Animals were killed by decapitation at 10 am to account for any variability in serum levels of testosterone. Epididymal and inguinal subcutaneous adipose tissues were immediately removed under sterile conditions.

Cell-culture

The stromavascular fraction was obtained after digestion of epididymal and femoral subcutaneous adipose tissues by collagenase as previously described (Pecquery *et al.*, 1983). The floating adipocytes were discarded and the infranatant containing the stromal vascular fraction was successively filtered through 150 μM and 25 μM nylon screens. The filtrate was centrifuged at 600 *g* for 10 min. After two washes in Dulbecco Modified Eagle Medium (DMEM) containing 8% fetal calf serum (FCS), HEPES (20 mM, streptomycin (0.1 mg/ml), penicillin (100 IU/ml) and sodium ascorbate (2.5 μg/ml), cells were inoculated in multi-well dishes (40 000 cells/cm^2) and maintained at 37°C under 5% CO_2 atmosphere. After plating, cells were extensively washed and maintained under the same conditions as above. When reaching confluence and for the same cell density (D5 and D6 for sub-cutaneous and epididymal cells, respectively), the cells were allowed to differentiate in DMEM/F12 (1:1) supplemented with HEPES (20 mM), biotin (33 μM), panthotenate (17 μM), insulin (5 μg/ml), transferrine (10 μg/ml), triiodothyronine (2 nM) sodium ascorbate (250 μg/ml) and antibiotics (streptomycine: 0.1 mg/ml and penicilline: 100 UI/ml) (Deslex *et al.*, 1987). Glycerol-3-phosphate dehydrogenase (G3PDH) was used as a marker of the stage of differentiation (Wise & Green, 1979). Eighty percent at least of the cells in culture were fully differentiated at day 6 post-confluence. Cell viability was assessed by trypan blue exclusion (Phillips, 1973).

AR assays

AR were studied by radioligand binding assays in confluent and differentiated cells. After removing the medium, cells were washed three times with DMEM and incubated (in triplicate) at 37°C, in DMEM containing different concentrations of [^{3}H]R1881 (methyltrienolone) and a fixed concentration (10 μM) of triamcinolone acetonide. Non-specific binding was measured under the same conditions except that 5α-dihydrotestosterone (1 μM) was added to the incubations. After 60 min, incubation medium was discarded and the cells were washed three times to remove the free steroids. Two different experimental approaches were then realized. First, a whole cell assay was performed as described by De Pergola *et al.*, 1990). Briefly, cells were solubilized with NaOH (0.2 M) and the radioactivity directly counted. An aliquot was removed for the assay of total cellular protein according to the method of Bradford (1976). Second, a nuclear binding assay was performed as described in (Pedersen *et al.*, 1991). The cells in monolayer were resuspended in cold buffer (1 M sucrose, 10% glycerol, 0.1% triton X100, 10 mM KCl and 50 mM Tris pH = 7.4). Precursor cells were homogenized on ice in a glass potter fitted with a teflon pestle and carefully layered over cold 1.4 M sucrose solution and centrifuged for 15 min at 2500xg. The supernatant was removed. The nuclear pellet was washed and resuspended in buffer containing Tris (50 mM), glycerol (10%), triton X100 (0.2%), albumine (0.1%) and the radioactivity was finally counted. DNA was quantified in nuclear preparation according to the method of Burton (1956).

Immunocytofluorimetry:

Preadipocytes were grown to semi-confluency on glass coverslips. At this state, cells were washed with phosphate buffered saline (PBS), fixed and permeabilized in phosphate buffer containaing 3% paraformaldehyde and 15% picric acid (Chang *et al.*, 1989) for 30 min at room temperature. After two washes with PBS, the monolayer cells were blocked with PBS containing 3% bovine serum albumine (BSA) for 20 min and then treated with a 1/1000 dilution of polyclonal rabbit anti-AR antibody (PA1-110) in PBS with 3% BSA, overnight, at 4°C. The specificity of PA1-110 has been validated previously for the study of rat androgen receptors (Takeda *et al.*, 1990; Clancy *et al.*, 1992). Negative controls were performed with a 1/1000 dilution of rabbit IgG or with the primary antibody preadsorbed with cytosol from rat prostate (Clancy *et al.*, 1992), under the same experimental conditions. The monolayer cells were then treated for 1 h at 37°C with the second antibody, a goat anti-rabbit IgG conjugated to fluorescein isothiocyanate (1/40 in PBS containing 3% BSA). After two washes with PBS, coverslips were mounted with citifluor on glass slides and viewed by conventional epifluorescence microscopy. Exposition time and photo processing were kept identical to allow valuable comparisons.

All results are expressed as means ± SEM of data obtained, at least, from three separate experiments. Statistical significance of the data was established using paired Student's *t* test.

Materials

[^{3}H]R1881 (specific activity: 86 Ci/mmol) was obtained from New England Nuclear Company, Dupont Les Ulis, France), triamcinolone acetonide, goat anti-rabbit IgG, DMEM, DMEM/F12, HEPES, biotin, panthotenate, porcine insulin, transferrine, triiodothyronine, FCS from Sigma chemical Co (St. Louis, MO), polyclonal rabbit anti-AR antibody (PA1-110) from Affinity Bioreagents (Neshanic Station, NJ, USA) and collagenase from Boehringer (Mannheim, Germany).

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References

Berthois, Y., Dong tue Fen, Roux-Dosseto, M. & Martin, P.M. (1990). *Mol. Cell. Endocrinol.*, **74,** 11–20.
Bjorntorp, P. (1991). *Int. J. Obesity*, **51,** 67–81.
Blok, L.J., Bartlett, J.M., Boltdevries, J., Themmen, A.P.N., Brinkmann, A.O., Weinbauer, G.F., Nieschlag, E. & Grootegoed, J.A. (1991). *J. Steroid. Biochem. Molec. Biol.*, **40,** 343–347.
Bonne, C. and Mayraud J.P. (1975). *Steroids*, **27,** 497–507.
Bradford, A.M. (1976). *Anal. Biochem.*, **72,** 248–254.
Burton, K. (1956). *Biochem. J.*, **62,** 315–323.
Chang, C., Whelan, C.T., Popovich, T.C., Kokontis, J. & Liao, S. (1989). *Endocrinology.*, **126,** 1097–1099.
Clancy, A.N., Bunsall, R.W. & Michael, R.P. (1992). *Life Sci.*, **50,** 409–417.
De Pergola, G., Xu, X., Yang, S., Giorgino, R. & Bjorntorp, P. (1990). *J. Steroid Biochem. Molec. Biol.*, **37,** 553–558.
Deslex, S., Negrel, R. & Ailhaud. (1987). *Exp. Cell. Res.*, **168,** 15–30.
Dieudonne, M.N., Pecquery, R. & Guidicelli, Y. (1992). *Eur. J. Biochem.*, **205,** 867–873.
Dieudonne, M.N., Pecquery, R., Leneveu, M.C., Dausse, J.P. & Giudicelli, Y. (1994). *Endocrine*, **2,** 567–570.
Freyschuss, B.O., Shavreus-evers, A., Sahlen, L. & Eriksson, H. (1993). *Endocrinology*, **133,** 1548–1554.
Gray, J.M. & Wade, G.N. (1980). *Am. J. Physiol.*, **239,** E237–E241.
Grino, P.B., Griffin, J.E. & Wilson, J.D. (1990). *Endocrinology*, **126,** 1165–1172.
Jaubert, A.M., Pecquery, R., Sichez, I., Dieudonne, M.N., Cloix, J.F. & Giudicelli, Y. (1993). *Endocrine*, **1,** 535–540.
Krongard, A., Wilson, C.M., Wilson, J,.D., Allman, D.R. & McPhaul, M.J. (1991). *Mol. Cell. Endocrinol.*, **76,** 79–88.
Krotkiewski, M., Bjorntorp, P., Sjostrom, L. & Smith, U. (1983). *J. Clin. Invest.*, **72,** 1150–1162.
Lacasa, D., Agli, B., Pecquery, R. & Giudicelli, Y. (1991). *Endocrinology*, **128,** 747–753.
Lacasa, D., Denis, D., Agli, B., De Mazancourt, P. & Giudicelli, Y. (1994). *Biochim. Biophys. Acta*, **1224,** 527–532.
Miller, L.K., Kral, J.G., Strains, G.W. & Zumoff, B. (1990). *Steroids*, **55,** 410–415.
Mizutani, T., Nishikawa, Y., Adachi, H., Enomoto, T., Ikegami, H., Nomura, T. & Miyake, A. (1994). *J. Clin. Endocrinol. Metab.*, **78,** 950–954.
Pecquery, R., Leneveu, M.C. & Giudicelli, Y. (1983). *Biochem. Biophys. Acta*, **731,** 397–405.
Pecquery, R., Leneveu, M.C. & Giudicelli, Y. (1988). *Endocrinology*, **122,** 2590–2596.
Pecquery, R., Dieudonne, M.N., Leneveu, M.C. & Giudicelli, Y. (1990). *Endocrinology*, **126,** 241–245.
Pecquery, R., Dieudonne, M.N., Cloix, J.F., Leneveu, M.C., Dausse, J.P. & Giudicelli, Y. (1995). *Biochem. Biophys. Res. Comm.*, **206,** 112–118.
Pedersen, S.B., Borglum, J.D., Eriksen, E.F. & Richelsen, B. (1991). *Biochim. Biophys. Acta*, **1093,** 80–86.
Phillips, H.J. (1973). Academic Press, NY. 406–408.
Price, T.M. & O'Brien, S.N. (1993). *J. Clin. Endocrinol. Metab.*, **77,** 1041–1045.
Prins, G.S. (1987). *Endocrinology*, **120,** 1457–1464.
Quarmby, V.E., Yarbrough, W.B., Lubahn, D.B., French, F.S. & Wilson, E.M. (1990). *Mol. Endocrinol.*, **4,** 22–28.
Rebuffe-Scrive, M., Enk, L., Crona, N., Lonnroth, P., Abrahamsson, L., Smith, U. & Bjorntorp, P. (1985). *J. Clin. Invest.*, **75,** 1973–1976.
Rebuffe-Scrive, M., Lonnroth, P., Marin, P., Wesslau, C., Bjorntorp, P. & Smith, U. (1987). *Int. J. Obesity*, **11,** 347–355.
Rebuffe-Scrive, M., Andersson, B., Olbe, L. & Bjorntorp, P. (1989). *Metabolism*, **38,** 453–458.
Roncari, D.A.K. & Van, R.L.R. (1978). *J. Clin. Invest.*, **62,** 503–508.
Rossini, G.P. (1991). *Biochem. Biophys. Res. Commun.*, **181,** 383–388.
Syms, A.J., Norris, T.S., Panko, W.B. & Smith, R.G., (1985). *J. Biol. Chem.*, **260,** 455–461.
Takeda, H., Chodah, G., Mutchnik, S., Nakamoto, T. & Chang, C. (1990). *J. Endocrinol.*, **126,** 17–25.
Wade, G.N., Gray, J.M. & Bartness, T.J. (1985). *Int. J. Obesity*, **9,** 83–92.
Wise, S. & Green H. (1979). *J. Biol. Chem.*, **254,** 273–275.
Xu, X., De Pergola, G., Eriksson, P.S., Fu, L., Carlsson, B., Yang, S., Eden, S. & Bjorntorp, P. (1993). *Endocrinology*, **132,** 1651–1657.