Beta-Adrenoceptor Expression in Human Fat Cells from Different Regions

Peter Arner,1 Lena Hellström,4 Hans Wahrenberg, and Mikael Brönnegård5

Departments of Medicine, Pediatrics, Medical Nutrition, and the Research Center Huddinge Hospital, S-141 86 Huddinge, Sweden

Abstract

The expression of beta-adrenoceptors (BAR) was investigated in abdominal and gluteal fat cells of 32 nonobese men and women using radioligand binding and RNA excess solution hybridization. In both sexes the number of BAR binding sites was about twice as high in abdominal as in gluteal fat cells (P < 0.01). Northern blot analysis of total RNA from adipose tissue showed hybridization of the BAR1 probe to an mRNA species of about 2.5 kb and of the BAR2 probe to an mRNA species of ~ 2.2 kb. The steady-state mRNA levels of BAR 1 and BAR 2 were also twice as high as in abdominal as in gluteal adipocytes of men and women (P < 0.01). In abdominal fat cells the mRNA levels were ~ 45 and 30 molecules/cell for BAR 1 and BAR 2, respectively. There were no regional or sex variations in BAR 1 and BAR 2 mRNA stability. The apparent half-life of mRNA for both receptor subtypes was ~ 6 h in both regions. The mRNA levels for beta actin did not differ between the two regions in either sex. Thus, differences in expression of the genes encoding for BAR 1 and BAR 2 can explain why abdominal fat cells have more BAR than gluteal fat cells. This variation in gene expression may be a molecular mechanism underlying the well known regional differences in catecholamine-induced lipolysis activity between central and peripheral adipose tissue. (J. Clin. Invest. 1990. 86:1595–1600.) Key words: beta-adrenoceptors • mRNA • catecholamines

Introduction

Catecholamines are the major lipolysis-promoting hormones in adult human fat cells (1). This effect is mediated by beta-adrenoceptors (BAR). In human fat cells, unlike in adipocytes from most other species, catecholamines also have antilipolytic properties that are mediated by alpha2-adrenoceptors. It is well established that there are marked regional variations in catecholamine-induced lipolysis; the hormones are much more lipolytic in abdominal than in gluteal/femoral human adipocytes (2–5). It has been suggested that site variations in lipolysis may be of importance for the difference in body fat distribution in men and in women and for the development of different types of regional obesity (6–8).

Address correspondence and reprint requests to Dr. Peter Arner, Department of Medicine, Huddinge Hospital, S-141 86 Huddinge, Sweden.

Received for publication 27 February 1990 and in revised form 5 June 1990.

1. Abbreviations used in this paper: BAR, beta-adrenoceptors; HCGP, [5,7-3H]benzimidazole-2-1 hydrochloride; [151]ICYP, [151]-cyano-pindolol; TNA, total nucelic acids.

0021-9738/90/11/1595/06 $2.00
Volume 86, November 1990, 1595–1600

The mechanisms underlying regional variations in lipolysis have been partly elucidated (9). It appears that an increase in the number of BAR (with normal affinity and normal coupling to lipolysis) is the major factor underlying an enhanced lipolytic effect of catecholamines in abdominal as compared with gluteal human fat cells. This may involve both BAR subtypes. The existence of BAR 1 and BAR 2 has been demonstrated in isolated human fat cells (10), and both receptor subtypes are coupled to lipolysis (11).

The factors responsible for variations in BAR content between fat cells from different adipose deposits are not known. Differences in gene expression, mRNA/protein processing, or protein degradation may be involved. In this study, we have investigated whether variations in BAR number between fat cells from different regions also involve differences in expression of the genes encoding for BAR 1 and BAR 2. Thus, steady-state mRNA levels for these two genes, as well as mRNA stability, were determined in abdominal and gluteal fat cells. mRNA was measured using solution hybridization techniques. The findings with mRNA have been related to cell surface BAR content in the adipocytes, which was determined by radioligand binding techniques.

Methods

Subjects. 32 healthy volunteers (17 men and 15 women) participated in the study. They were all drug free. None of the women had taken oral contraceptives during the preceding 6-mo period. Their ages were 23–55 yr (mean±SE:33±2 yr). All were of normal weight and the body mass index was 24.0±0.3 kg/m2. All subjects gave their informed consent and the study was approved by the Ethics Committee of Huddinge Hospital. After the subjects had fasted overnight, one subcutaneous specimen (2–3 g) was excised from the infraumbilical region (abdominal site) and one from the upper lateral quadrant of the gluteal region under local anesthesia, as described previously (9). A part of the tissue was immediately frozen in liquid nitrogen and stored at ~ −70°C. Fat cells were isolated from the remaining part as described previously (12). Some of these cells were frozen and stored as above. The remaining cells were used for receptor binding, as described below.

Receptor binding assays. Incubations were carried out under steady-state conditions, as described in detail elsewhere (12). Briefly, ~ 20,000 fat cells were incubated in triplicate at 37°C in 1 ml of Krebs-Ringer phosphate buffer (pH 7.4) containing 5 g/liter bovine serum albumin, 1 g/liter glucose, and 0.1 g/liter ascorbic acid plus radiogand

In most of the experiments one low and one high concentration of radiogand was used. It was possible to perform complete saturation experiments in only six of the subjects because the amounts of adipose tissue available were limited. The hydrophobic antagonist [125]ICYP was used in the saturation experiments and the hydrophilic antagonist (±)4-(3-butylamino-2-hydroxypropoxy)−[5,7-3H]benzimidazole-2-1 hydrochloride ([HCGP] was used in the other experiments. We have repeatedly shown (9, 13) that these radioligands bind to a single class of homogeneous, noninteracting binding sites that give straight lines on a Scatchard analysis and Hill coefficients near 1. Under such conditions, the use of two radioligand concentrations to determine antagonist-binding capacity seems reliable, because both binding points will be distributed along the same regression line.

Beta-Adrenoceptor Expression 1595
Adipocyte measurements. The fat cell volume and the number of fat cells incubated were determined as described previously (10).

Preparation of nucleotides. For solution hybridization analysis, total nucleic acids (TNA) were prepared by digestion of homogenized adipose tissue or fat cells with proteinase K in an SDS-containing buffer (1% SDS; 10 mmol/liter of Tris, pH 7.5; 5 mmol/liter of EDTA; protein kinase K, 100 mg/liter) and subsequent extraction with phenol-chloroform by the addition of isomylc ethanol (vol 24:1) (14). The concentration of nucleic acids was measured spectrophotometrically.

Northern blot analysis required ∼ 20 g of subcutaneous adipose tissue, which was obtained during elective cholecystectomy on two male nonobese subjects (ages 47 and 50, respectively), but were otherwise healthy. The tissue was extracted as described above. Total RNA was prepared by the acid quainidum thiocyanate method combined with the CsCl centrifugation procedure (15). The amount of RNA was estimated from the optical density at 260 nm.

Preparation of hybridization probes. Complementary oligonucleotide probes (50 bases) were synthesized and hybridized to each other in a procedure described by Melton et al. (16). In addition, the hybrid contained recognition sequences for the restriction enzymes Pst I and Hind III. The constructions were cloned into the Pst I/Hind III sites in pGEM-3 (Promega Biotec., Madison, WI). The sequences of these insertions were confirmed by DNA sequencing using the dyeoxy chain-termination method (17). The plasmid was employed for in vitro synthesis of cRNA using SP6 RNA polymerase, and for the opposite mRNA strand using T7 RNA polymerase. For solution hybridization analysis, the probes were radiolabeled with [32P]UTP, and for Northern blot analysis [32P]UTP was used. The sequence of the oligonucleotides used was for the beta-1-adrenergic receptors (nucleotides 739-789) (18), 5'GTG ATC GAC TGC GAG GCC CAG AGG CAG GTG AAC AAG ATC GAC AGC TGC GAG CGC 3'; and for the beta-2-adrenergic receptor, (nucleotides 772-822) (19), 5'CAG AGA TCT TCC TTC GGC GAC CAC AAC CAG GTG AGG CAG CAC AAG GCC CTC AAG ACG 3'. As a methodological control we employed a [32P]-labeled CDNA probe encoding the beta-actin (20).

Northern blot hybridization. This assay was performed essentially as described by Maniatis et al. (7). RNA samples (20-30 μg) were denatured for 10 min at 65°C in dimethylsulphoxide (50%), formaldehyde (2.2 mol/liter), sodium phosphate (10 mmol/liter, pH 7.5), EDTA (0.05 mmol/liter), and then electrophoresed through an agarose gel (1.2%) containing formaldehyde (2.2 mol/liter) in sodium phosphate (10 mmol/liter, pH 7.5) for 500 V-h in a running buffer containing morpholinopropane sulphonic acid (0.04 mmol/liter), and collected on glass fiber filters (Whatman Inc., Clifton, NJ). Sample TNA hybridization was compared with a known amount of an in vitro synthesized mRNA strand (complementary to the radioactive probe). A standard curve (0.5-30 × 10^-4 mol mRNA/incubation) was included in each assay. Each TNA sample was analyzed in duplicate and the amount of the BAR1 mRNA or BAR2 mRNA was related to the amount of DNA in the TNA sample. Results are expressed as molecules/cell, assuming a content of 6.4 pg of DNA/adipocyte.

Statistical methods. The values presented are means±SE. Regression analysis, the F-distribution t test for comparing two regression lines and the paired and unpaired t tests were used for statistical comparison of the results.

Results

Adipose cellularity. Adipocytes from the gluteal region were larger than those from the abdominal region, the adipocyte volume being 572±27 pl vs. 453±27 pl (P < 0.001). Moreover, the fat cells from males were somewhat smaller than those from women in both regions. These data confirm the results from several laboratories, including a recent study done by us (9).

Radioigand binding. The binding of labeled beta-adrenoceptor ligand was significantly increased in abdominal adipocytes as compared with gluteal adipocytes (Fig. 1). This was true only when two concentrations of the radioligand 2HCGP were used (in all subjects) as well as in the saturation experiments with 2HICYP binding (six subjects). If the results using the two different concentrations of each radioligand are representative of complete saturation experiments, they indicate that the number of beta-adrenoceptor binding site is almost twice as high in abdominal adipocytes as in gluteal adipocytes. Moreover, the Scatchard analysis of complete saturation experiments with 2HICYP displayed straight and parallel lines for binding to both abdominal and gluteal cells. This shows that there was no difference between the two sites in adenoreceptor affinity for the radioligand. The Scatchard analysis also demonstrated that the maximum binding capacity of 2HICYP was almost twice as high in abdominal as in gluteal adipocytes (P < 0.001). Thus, there was an almost twofold difference between abdominal and gluteal cells in the number of beta-adrenoceptors. The sex of the subjects had no significant effect on beta-adrenoceptor binding (data not shown). The binding data were expressed per cell number. The regional differences in binding were even more marked when expressed per cell surface area. The findings with BAR binding confirm previous results from our laboratory concerning gluteal and abdominal human adipocytes (9).

Measurements of mRNA. In this study we used cRNA probes derived from synthetic oligonucleotides (50 bases) in order to quantify specific BAR 1 and BAR 2 mRNA species. The sequences of the probes were obtained from published sequences and were confirmed by DNA sequencing. The hybridization conditions in the assay were determined from an analysis of the melting point of RNA-RNA hybrids (data not
In methodological studies, mRNA for beta-actin was measured in adipose segments of six men and six women. In women, the values (molecules/cell) were 75±8 and 73±8 in the abdominal and gluteal regions, respectively. The corresponding values in men were 69±9 and 76±12. Thus, beta-actin mRNA showed no sex or regional variation.
In order to determine if there was any sex difference in gene expression of BAR 1 and BAR 2, we compared mRNA in men and women. Fig. 5 shows the results with isolated fat cells. In both regions, the values for the two BAR mRNA species for men and women were almost identical. Similar results were found when adipose tissue segments were analyzed (data not shown).

In Figs. 4 and 5 the mRNA content was related to DNA (i.e., molecules/cells). In uncharted experiments mRNA was also related to TNA and is expressed as mol of mRNA/μg of TNA. These results were essentially the same as those obtained when DNA was used as the denominator.

In order to determine whether there existed any regional differences in mRNA stability, adipose tissue segments were incubated in vitro with the RNA polymerase inhibitor actinomycin D (Fig. 6). mRNA for BAR 1 and BAR 2 declined as a function of time (semilogarithmic relationship). However, there was no difference in the rate of decline between the two mRNA species or between regions. The apparent half-life of both mRNA species was ~ 6 h.

Discussion

In this study, the possible functional importance of BAR gene expression has been investigated for the first time in man. The expression of receptor protein may be regulated at both the transcriptional and the posttranscriptional levels. The latter includes effects on mRNA stability, translation efficiency, protein turnover, or a modification of the protein that alters its biological activity.

Our study shows that the BAR receptor number is higher in abdominal than in gluteal adipocytes of men and women. This is in consonance with our earlier study (9). Since we also measured mRNA levels for the genes that encode for BAR, it is possible to understand the molecular mechanisms that may be responsible for the variations in BAR expression between different types of adipocytes for the first time.

The steady-state mRNA levels for BAR 1 and for BAR 2 were about twice as high in abdominal as in gluteal fat cells. This corresponded well with the twofold difference in cell surface BAR number between the two cell types that were also observed in this study. Because of the limited amount of tissue available, we could not determine the proportions of BAR 1 and BAR 2 binding sites in the two adipose regions. Therefore, it is not known at present whether regional differences in BAR number involve both receptor subtypes or only one receptor subtype. Basal mRNA levels of BAR have previously proved to be in agreement with the levels of these receptor subtypes, as determined by radioligand binding techniques in different rat tissues (18). This study indicates that measurement of BAR mRNA levels by solution hybridization in human adipose tissue accords well with determination of BAR by ligand binding. Thus, the observed increase in BAR mRNA expression in abdominal adipocytes may be the major reason why these cells have a higher number of cell surface BAR than gluteal adipocytes have.

It is unlikely that the site difference in BAR mRNA is due to a general increase in mRNA in abdominal tissue as compared with gluteal tissue, since the amounts of beta-actin mRNA were almost identical in cells from both regions. Moreover, the regional variations in BAR mRNA cannot be attributed to stroma cells, as similar results were obtained with adipose segments and with isolated fat cells.

The site differences in BAR mRNA levels can scarcely be due to variations in mRNA stability. The apparent half-lives of BAR 1 and BAR 2 mRNA in segments of isolated adipose tissue were similar in both regions, as evidenced by the results of experiments using actinomycin D. These results may indicate that site variations in BAR mRNA levels are due to differences in the gene transcription rate. However, the mRNA half-life is only an indirect indicator of transcription. In addition, actinomycin D in itself may influence mRNA metabolism. Unfortunately, it was not possible to directly measure the transcription rate of the BAR genes in the small amount of human adipose tissue that was available in our study.

It has been demonstrated earlier that regional differences in catecholamine-induced lipolysis are more apparent in women than in men (5, 9). There are, however, no sex variations in
beta-adrenoceptor-mediated lipolytic effects in fat cells from the two regions (9). This agrees with the present findings that show no influence of sex on BAR number or BAR 1 and BAR 2 mRNA expression.

Adenylate cyclase-linked receptors, such as the BAR subtype, are low abundance proteins with low basal levels of corresponding mRNAs (22, 23). Therefore, it is not possible to measure these mRNA levels in small amounts of human adipose tissue with the standard quantitative Northern blot analysis because this method has low sensitivity. In this study, we measured mRNA levels using a very sensitive solubilization hybridization assay. Previously, BAR 2 mRNA has been determined in hamster DDT1 MF-2-cells using this technique (22, 23). The steady-state levels of BAR 2 mRNA appear to be in the same order of magnitude in these hamster cells as in human fat cells.

Although BAR1 and BAR2 are encoded by different genes, previous studies have indicated similarities among BAR subtypes in immunological and photoaffinity labeling properties as well as in the SDS-polyacrylamide gel electrophoresis migration pattern (MW, 65,000-67,000) for both receptor subtypes (24). These data suggest that the molecular basis for the subtype specificity of BAR may be due to posttranslational modification. However, studies by Babouth et al. (25) and Strader et al. (26), using a cDNA probe for the hamster BAR2, indicated that posttranslational modification of the receptor by a cell in which it is expressed does not dictate subtype specificity. We also found that the size of the mRNA species encoding for the BAR1 (2.5 kb) was larger than that encoding for the BAR2 (2.2 kb). This confirms previous results in other tissues (18, 19) and indicates an important structural difference between the two mRNA species in human fat cells. Thus, it seems likely that in human fat cells the subtype character of the BAR is the result of the expression of separate but homogenous genes. Recently, a BAR 1 gene has been isolated in man (27). Whether this gene is also expressed in human fat cells remains to be established.

Our results indicate that the specific regulation of BAR subtype expression in human adipose tissue involves transcriptional rather than posttranscriptional mechanisms. The results of recent studies indicate that hormones which are important for fat cells, such as catecholamines and glucocorticoids, can alter transcription of BAR genes resulting in corresponding changes of the steady-state BAR mRNA levels (28, 29). Thus, the presently observed regional difference in mRNA levels for BAR1 and BAR2 may be due to variations in the action of hormones or other regulatory agents on BAR gene transcription in adipocytes from different fat depots. However, posttranscriptional regulation of BAR protein expression in fat cells must be considered as well. It has recently been demonstrated that catecholamines have posttranscriptional effects on BAR mRNA levels. These hormones seem to promote destabilization of mRNA (30).

In fat cells of either sex or region, the basal BAR1 mRNA levels were ~50% higher than the BAR2 mRNA levels. This was not due to any differences in RNA stability, as judged by the experiments with actinomycin D. The latter may indicate that there is a variation in gene transcription activity between the BAR subtypes in human fat cells. The functional importance of the observed difference in mRNA expression of BAR1 and BAR2 is not known at present, since reasons discussed previously, we were not able to determine the number of each receptor subtype in this study. However, previous studies from our laboratory suggest that the proportion of BAR1 relative to BAR2 binding sites in intact human fat cells is 4 to 1 (10). We have also found that the lipolytic order of potency for catecholamines in isolated human fat cells indicates a preponderance of BAR1 (31). Thus, previous functional studies from our own laboratory regarding the two BAR subtypes are in agreement with the present mRNA data.

In the Northern blot analyses (Fig. 2), the mRNA signals for BAR1 and BAR2 were of similar strengths, which may seem at odds with the solution hybridization data. It should, however, be stressed that the Northern blot experiments were not designed to be quantitative. Instead they were used to test the specificity of the probes and to investigate the size of each mRNA species. Neither the amount of RNA that was blotted nor the hybridization conditions were standardized in the rigorous way that is necessary for quantitative Northern blot analysis.

In summary, abdominal adipocytes have more BAR binding sites than do fat cells from the gluteal area in men and women. This can be explained by an increased expression of the genes that encode for BAR 1 and BAR 2 in the former cells. This difference in gene expression constitutes a molecular mechanism for regional variations in adipocyte lipolysis activity.

Acknowledgments

This study was supported by grants from the Swedish Medical Research Council (19K-08714), Draco AB (Lund, Sweden), the Swedish Diabetes Association, Swedish Athlets Research Council, Swedish Medical Association, Swedish Cancer Society, Karolinska Institute, and the Foundations of Osterman, Nordic Insulin, Stohne, and Old Female Servants.

References


Beta-Adrenoceptor Expression


