British Journal of Pharmacology (1996) 118, 929-934

Respective degree of expression of β_1-, β_2- and β_3-adrenoceptors in human brown and white adipose tissues

*Chengjun Deng, *Ariane Paoloni-Giacobino, *Françoise Kuehne, *Olivier Boss, *Jean-Pierre Revelli, *Madelaine Moinat, [1]†Michael A. Cawthorne, *Patrick Muzzin & [2]*Jean-Paul Giacobino

*Département de Biochimie Médicale, Centre Médical Universitaire, 1211 Genève 4, Switzerland. †Department of Vascular Biology, SmithKline Beecham Pharmaceuticals, Epsom, Surrey KT18 5XQ, U.K.

1 The possible existence of a β_3-adrenoceptor in human brown and white adipose tissues was investigated by mRNA expression and binding studies.

2 The relative amounts of β_1-, β_2- and β_3-adrenoceptor mRNA, as determined by total RNA Northern blot analysis in newborn brown adipose tissue, were 28, 63 and 9% respectively of the total β-adrenoceptor mRNA.

3 The β_1/β_2-adrenoceptors of human brown adipose tissue plasma membranes were characterized using [^{3}H]-CGP 12177 as a ligand. Their K_d and B_{max} values were 1.9 nM and 156 fmol mg^{-1} of membrane proteins, respectively. The β_3-adrenoceptor was characterized by use of the new specific radioligand [^{3}H]-SB 206606. The binding of this ligand was stereospecifically displaced by the active **R,R**- or the inactive S,S-enantiomer of BRL 37344 up to a concentration of about 10 μM. The K_d and B_{max} values of the brown adipose tissue membrane β_3-adrenoceptors were 87 nM and 167 fmol mg^{-1} of proteins, respectively. A low affinity [^{3}H]-CGP 12177 binding site population was also detected in these membranes.

4 In human omental white adipose tissue, no β_3-adrenoceptor mRNA could be detected in total RNA Northern blots and the β_1- and β_2-adrenoceptor mRNAs represented 9 and 91%, respectively of the total β-adrenoceptor mRNA, and no specific binding of [^{3}H]-SB 206606 could be measured.

Keywords: β-Adrenoceptors; human adipose tissue; mRNA expression; ligand binding

Introduction

Before the β_3-adrenoceptor was identified, the β-adrenoceptor population of the rodent brown adipose tissue had been reported to consist mostly of the β_1-adrenoceptor subtype (Rothwell *et al.*, 1985; Levin & Sullivan, 1986; Muzzin *et al.*, 1988) and that of the white adipose tissue, i.e. of the mature 3T3-L1 adipocyte and of the human abdominal subcutaneous fat, mostly of the β_2-adrenoceptor subtype (Lai *et al.*, 1982; Mauriège *et al.*, 1988).

The gene for the β_3-adrenoceptor was first cloned from a human genomic library (Emorine *et al.*, 1989) and then from rat brown adipose tissue cDNA (Muzzin *et al.*, 1991; Granneman *et al.*, 1991) and mouse genomic (Nahmias *et al.*, 1991) libraries. In the rodents, the β_3-adrenoceptor was found to be strongly expressed in brown and white adipose tissues (Muzzin *et al.*, 1991; Granneman *et al.*, 1991; Nahmias *et al.*, 1991), suggesting that it might represent a fat cell-specific β-adrenoceptor subtype. It was also identified in gut tissues both from functional (Arch & Kaumann, 1993) and molecular biology (Bensaid *et al.*, 1993) studies. The observation that, in fact, three β-adrenoceptor subtypes were co-existing in the adipocyte membrane was an incitement to quantify the relative amount of each β-adrenoceptor subtype in terms of mRNA and receptor protein. In mature 3T3-L1 adipocytes and in rat brown adipose tissue, the β_3-adrenoceptor was found, by binding studies, to be the predominant β-adrenoceptor subtype (Fève *et al.*, 1991; Muzzin *et al.*, 1992). The same finding was made in rat epididymal white adipose tissue (unpublished observation). The level of the β_3-adrenoceptor mRNA was, like that of the β_3-adrenoceptor protein, reported to predominate in rat brown adipose tissue (Muzzin *et al.*, 1991; Granneman & Lahners, 1992).

The question of whether the β_3-adrenoceptor is expressed in human tissue was until recently unanswered. In 1993 Krief *et al.*, using reverse transcription polymerase chain reaction (RT-PCR) analysis, detected β_3-adrenoceptor mRNA in infant perirenal brown adipose tissue, in various deposits of adult white adipose tissue and in gallbladder. Studies in our laboratory (Revelli *et al.*, 1993) and in that of Granneman *et al.* (1993), using the same technique, confirmed the existence of β_3-adrenoceptor mRNA in adult human white adipose tissue. The existence of β_3-adrenoceptor mRNA signal(s) was also revealed in infant brown adipose tissue by Northern blot (Krief *et al.*, 1993) and by nuclease protection (Granneman, 1995). In none of these studies, however, was an attempt made to quantify the relative amount of the β_3-adrenoceptor mRNA as compared to those of the β_1- and β_2-subtypes.

Functional studies measuring the lipolytic response of human white adipose tissues to β_3-adrenoceptor agonists have produced conflicting results (Giacobino, 1995). A recent paper of Enocksson *et al.* (1995) shows a clear lipolytic response to the specific β_3-adrenoceptor agonist, CGP 12177, of human subcutaneous abdominal adipose tissue *in vivo*.

Concerning binding studies, attempts were made using the classical β-adrenoceptor radioligand [^{125}I]-iodocyanopindolol (ICYP) in human white adipose tissue membranes, but the results could not be interpreted because the non-specific binding was too high (Langin *et al.*, 1991; Lönnqvist *et al.*, 1993). Experiments performed with another β-adrenoceptor ligand [^{3}H]-CGP 12177 (Revelli *et al.*, 1993) allowed the detection in adult omental fat membranes of low affinity [^{3}H]-CGP 12177 binding sites different from the β_1/β_2 binding sites, which were hypothesized to be β_3-adrenoceptors. Recently, the **R,R**-enantiomer of the β_3-adrenoceptor agonist, BRL 37344 was tritiated and the new radioligand obtained, [^{3}H]-SB 206606, was characterized (Muzzin *et al.*, 1994). The K_d values for [^{3}H]-SB 206606 binding and the K_i values for various β-adrenoceptor agonists and antagonists were found to be quite

[1]Present address: Clore Laboratory, University of Buckingham, Hunter Street, Buckingham MK18 1EG, U.K. [2]Author for correspondence.

comparable in membranes from Chinese hamster ovary K1 cells transfected with the rat β_3-adrenoceptor and from rat brown adipose tissue. These similarities were interpreted as indicating that, in a complex membrane system, [^{3}H]-SB 206606 is a valuable ligand for measurement of specific interactions with the β_3-adrenoceptor (Muzzin *et al.*, 1994).

The first aim of the present study was to determine by Northern blot analysis, the respective levels of the three β-adrenoceptor subtype mRNAs in human brown and white adipose tissues and the second was, using both [^{3}H]-CGP 12177 and the new β_3-adrenoceptor radioligand, [^{3}H]-SB 206606, to quantify the β_3-adrenoceptor in human brown and white adipose tissue membranes.

Methods

Human tissue

Pieces of human omental adipose tissue weighing between 10 and 40 g were obtained during intra-abdominal surgery and immediately frozen in liquid nitrogen. The patients were men below the age of 65 and were operated on for cancer, inflammatory bowel disease or cholecystectomy. Their body mass index was 23 ± 1 kg m^{-2}, i.e. in the normal range. No patient had any identified metabolic or endocrinological disorder. Pieces of perirenal brown adipose tissue weighing about 1.5 g were obtained during renal surgery from male or female patients (mean age: 3 months). Subjects had been fasted for at least 12 h; general anaesthesia was obtained with isoflurane or enflurane. The project had been approved by the Ethics Commission of the Department of Surgery of the Geneva Faculty of Medicine. For some binding assays, perirenal brown adipose tissue obtained from autopsies was used with the authorization of the Department of Pathology of the Geneva Faculty of Medicine (mean age: 1 month; mean post mortem time: 8 h). No difference in binding parameters could be observed between tissue obtained during surgery or from autopsy.

Northern blots

Total human omental adipose tissue RNA was isolated by the caesium/trifluoroacetic acid gradient method of Okayama *et al.* (1987). Total RNA (20 μg) was electrophoresed in a 1% agarose gel containing formaldehyde, as described by Lehrach *et al.* (1977), and transferred to Hybond N membranes by capillary blotting. Human β_2-adrenoceptor cDNA (Chung *et al.*, 1987) and human genomic β_1- and β_3-adrenoceptor probes (Revelli *et al.*, 1993) with sizes of 1.2, 1.2 and 0.9 kb, respectively, were labelled by random priming with [α-^{32}P]-dCTP to a specific radioactivity of approximately 1×10^9 d.p.m. μg^{-1} DNA. RNA blots were hybridized for 1 h at 68°C in Quik-Hyb, then washed in a solution of $0.1 \times$ SSC ($1 \times$ SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0)/0.1% sodium dodecyl sulphate at 60°C for 15 min and exposed to Kodak X-AR film at −70°C. Size estimates for the RNA species were established by comparison with an RNA ladder. Densitometric analysis of autoradiograms was performed with a high-resolution laser-beam densitometer. In all densitometric measurements, the amount of RNA electrophoresed was within a range in which the intensity of the bands were found to increase linearly with the amount of RNA. Student's unpaired *t* test was used to determine statistical significance.

Adipocyte membrane preparation and binding studies

Isolated adipocytes of human omental adipose tissues were prepared according to the method of Rodbell (1964). Immediately after fat cell isolation, adipocyte ghosts were prepared by lysis as described by Hollenga *et al.* (1991) and the crude membranes obtained by centrifugation at 105,000 *g* for 1 h. Newborn brown adipose tissue plasma membranes were prepared as previously described (Giacobino, 1979). Protein concentrations were determined by the method of Lowry *et al.* (1951) with BSA used as standard. The conditions chosen to study the binding of [^{3}H]-CGP 12177 and [^{3}H]-SB 206606 to human adipocyte membranes were those determined in a previous study with rat brown adipose tissue (Muzzin *et al.*, 1994). Briefly, the membranes were incubated for 30 min at 37°C in 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$ (total volume 0.5 ml) containing the indicated concentrations of [^{3}H]-SB 206606. GTP (50 μM) was systematically added to the incubation medium in order to obtain only one conformational state of the receptor. The binding of the radioligand to the membranes was determined by filtration using a Brandel M-24 R apparatus. Each assay was performed in duplicates. Specific binding was defined as described under Results. K_i, K_d and B_{max} values were calculated by Scatchard analysis using the LIGAND programme (Munson & Rodbard, 1980).

Chemicals

All organic and inorganic chemicals were of analytical or molecular biology grade and were purchased from Merck (Darmstadt, Germany), Sigma (St-Louis, MO., U.S.A.), Fluka (Buchs, Switzerland), Gibco BRL (New York, NY, U.S.A.) or Boehringer (Mannheim, Germany). ((-)-4-(3-*t*-Butylamino-2-hydroxypropoxyl)-[5,7-^{3}H]benzimi-dazol-2-one) i.e. (-)- [^{3}H]-CGP 12177 (30 Ci mmol^{-1}), [α-^{32}P]-dCTP (3000 Ci mmol^{-1}) and Hybond N membranes were purchased from Amersham (Bucks., U.K.). The Quik-Hyb hybridization mix was from Stratagene (La Jolla, CA, U.S.A.) and Ready Protein scintillation cocktail from Beckman (Paolo Alto, CA, U.S.A.). **R,R**- and **S,S**-enantiomers of BRL 37344 (methyl-2,6-diiodo-4-[(2-(2-(3-chlorophenyl)-2-hydroxyethyl) amino) propyl] phenoxy acetate) (BRL 44092A and 45182A, respectively) and [^{3}H]-SB 206606 ((**RR**)-4-[(2-2-(3-chlorophenyl)-2-hydroxyethyl) amino)-propyl[^{3}H]phenoxyacetate) were gifts from SmithKline Beecham Pharmaceuticals (Epsom, U.K.).

Results

Analysis of Northern blots performed with total RNA and hybridized with β_1-, β_2- and β_3-adrenoceptor DNA probes allowed for the semi-quantification of the relative amount of the three different β-adrenoceptor subtypes in human adipose tissue. It can be seen in Figure 1 that, in newborn perirenal brown adipose tissue, the intensities of the mRNA signals are $\beta_2 > \beta_1 > \beta_3$-adrenoceptor. In adult omental adipose tissue, the β_2-adrenoceptor mRNA signal is largely predominant over the $\beta_1$adrenoceptor mRNA signal, and the β_3-adrenoceptor mRNA signal is not detectable. The sizes of the transcripts are, as previously described (Revelli *et al.*, 1993), of 3.1 and 2.1 kb for β_1- and β_2-adrenoceptor mRNA, respectively and 2.3 and 2.8 kb for the β_3-adrenoceptor mRNA. Analysis of the autoradiograms by densitometry allowed the determination of the respective amount of each subtype (Table 1). It can be seen from the standard errors that the results were quite reproducible. The β_3-adrenoceptor was found to be 7 times, and at least 100 times less expressed than the β_2-adrenoceptor in brown and white adipose tissue, respectively. In brown adipose tissue the intensity of the 2.3 kb β_3-adrenoceptor signal was 41% that of the 2.8 kb signal. It is noteworthy that in a previous study using more sensitive techniques, i.e. RT-PCR and poly (A)$^+$ RNA Northern blots (Revelli *et al.*, 1993), a β_3-adrenoceptor mRNA could be detected in human omental adipose tissue.

Figure 2 shows a saturation kinetic analysis of [^{3}H]-CGP 12177 binding to newborn brown adipose tissue plasma membranes. Scatchard analysis of individual saturation isotherms using the LIGAND programme (Munson & Rodbard, 1980) indicated the presence of two populations of binding sites (inset of Figure 2). The K_d and B_{max} values of the high affinity [^{3}H]-CGP 12177 binding sites, which might represent the β_1/β_2-adrenoceptor population, and of the low affinity [^{3}H]-

CGP 12177 binding sites are shown in Table 2. Figure 3 shows a saturation kinetic analysis of [^{3}H]-SB 206606 binding to newborn brown adipose tissue plasma membranes. The non-specific binding value was measured for each concentration of [^{3}H]-SB 206606 by adding a concentration of **R,R**-BRL 37344 200 fold that of the labelled ligand as determined from competition studies (Figure 4). Scatchard analysis of individual saturation isotherms using the LIGAND programme (Munson & Rodbard, 1980), indicated the presence of one population of binding sites (inset of Figure 3) the K_d and B_{max} values for which are shown in Table 2.

Competition studies were then performed with a concentration of [^{3}H]-SB 206606 close to the K_d value determined by saturation kinetic analysis (Table 2), i.e. 50 nM and increasing concentrations of the active **R,R** or the inactive **S,S**-enantiomers of BRL 37344. As shown in Figure 4, [^{3}H]-SB 206606 binding to newborn brown adipose tissue plasma membranes is displaced stereospecifically by BRL 37344 up to a concentration of about 10 μM i.e. 200 fold that of the labelled ligand. At higher concentrations, [^{3}H]-SB 206606 binding is further displaced but non-stereospecifically by BRL 37344. The specific binding of [^{3}H]-SB 206606 to newborn brown adipose tissue plasma membranes can be defined as the part of the total binding which can be displaced stereospecifically. The inset of Figure 4 shows that in human omental white adipose tissue membranes, it was not possible to detect any specific binding of [^{3}H]-SB 206606.

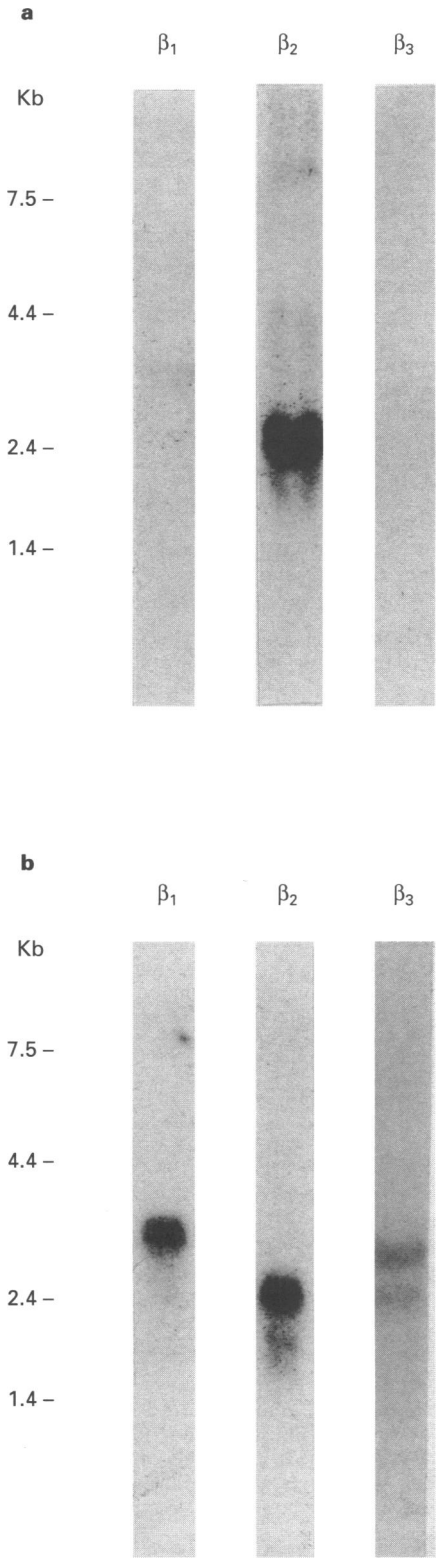

Figure 1 β-Adrenoceptor (β-AR) subtype mRNA signals in human adult omental adipose tissue (a) and in newborn perirenal brown adipose tissue (b): 20 μg of total RNA was electrophoresed, transferred to membrane filters and hybridized with ^{32}P-labelled human β_1-, β_2- and β_3-AR DNA probes of similar specific activities. The figure shows representative autoradiograms from the same patient. The duration of exposure of hybridized Northern blots to Kodak X-AR films was 2–4 days. The positions of molecular size markers are shown in Kb (0.24–9.5 Kb RNA ladder).

Table 1 Relative amounts of β_1-, β_2- and β_3-adrenoceptor (AR) mRNA in human newborn perirenal brown adipose tissue and adult omental white adipose tissue determined by Northern blot analysis

	mRNA level (% of total)	
	Brown fat	*White fat*
β_1-AR	28 ± 4 (6)	9 ± 3 (6)
β_2-AR	63 ± 5 (6)	91 ± 3 (6)
β_3-AR	9 ± 2 (6)	ND

Total RNA (20 μg) was electrophoresed, transferred to membrane filters and hybridized with ^{32}P-labelled human β_1-, β_2- and β_3-AR DNA probes of similar specific activities for the same period of time. Autoradiograms were analyzed by densitometry as described under Methods. The results are expressed as percentage of the sum of the 3 β-AR subtype signals. The values are the mean ± s.e. of the results obtained from the number of patients indicated in parentheses. ND = non detectable.

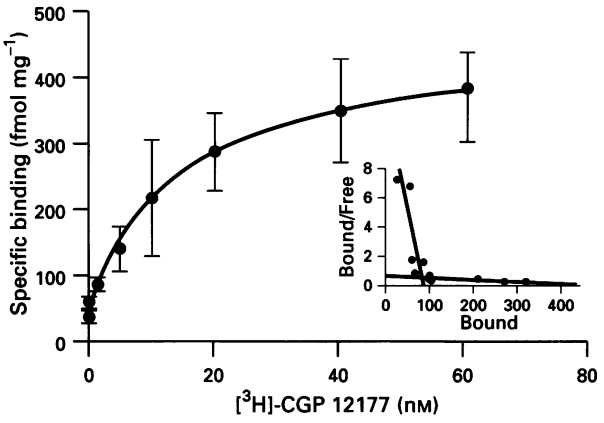

Figure 2 Specific binding of [^{3}H]-CGP 12177 to human newborn brown adipose tissue plasma membranes as a function of increasing concentrations of the ligand. The specific binding values shown are the difference between the total binding value obtained in the absence of competing ligand and the non-specific binding value obtained in the presence of (-)-propranolol (100–500 μM). The results illustrated are the mean ± s.e. of 3 experiments, each corresponding to a different patient, and are expressed as fmol of ligand bound mg^{-1} of cell membrane proteins. Inset: Scatchard analysis of the data of a representative experiment.

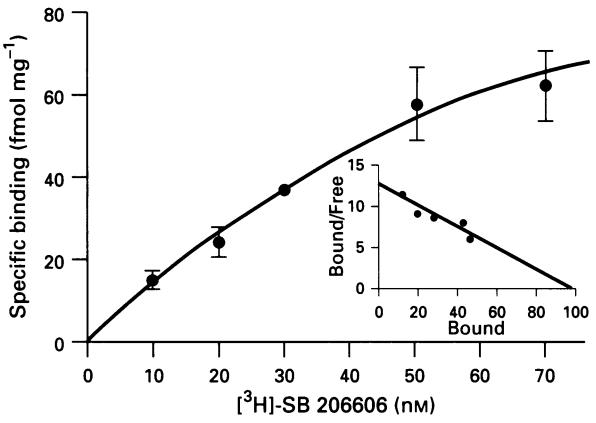

Figure 3 Specific binding of [^{3}H]-SB 206606 to human newborn brown adipose tissue plasma membranes as a function of increasing concentrations of the ligand. The specific binding values shown are the difference between the total binding value obtained in the absence of competing ligand and the non specific binding value obtained in the presence of concentration of **R,R-** BRL 37344 200 fold those of [^{3}H]-SB 206606. The results illustrated are the mean ± s.e. of 4 experiments and are expressed as fmol of ligand bound mg^{-1} of cell membrane proteins. Inset: Scatchard analysis of the data of a representative experiment.

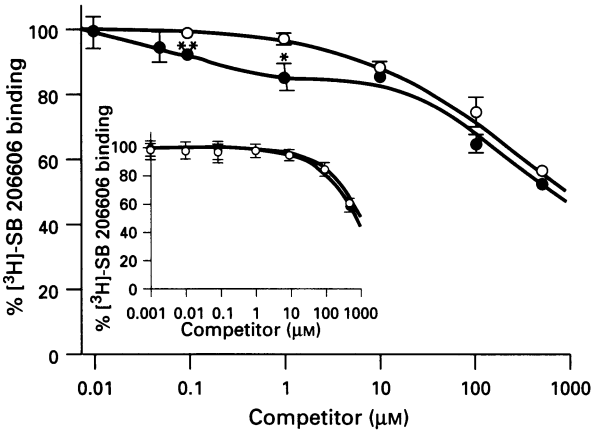

Figure 4 Displacement of [^{3}H]-SB 206606 50 nM binding to newborn brown adipose tissue plasma membranes by increasing concentrations of the **R,R-** (●) or **S,S-** (○) enantiomer of BRL 37344. The results illustrated are the means ± s.e. of 5 experiments and are expressed as a percentage of the total binding value in the absence of competitor. Inset: Displacement of [^{3}H]-SB 206606 50 nM binding to human omental fat adipocyte membranes by increasing concentrations of the **R,R-** or **S,S-**enantiomer of BRL 37344. The results are the means ± s.e. of 4 experiments; $*P<0.025$; $**P<0.01$ as compared to the values obtained in the presence of **S,S-**BRL 37344.

If it is considered that in human brown adipose tissue the high affinity [^{3}H]-CGP 12177 binding sites represents the β_1/β_2-adrenoceptors and the [^{3}H]-SB 206606 binding sites the β_3-adrenoceptor, it can be calculated that the β_3-population amounts to 52% of the total β-adrenoceptors in this tissue.

Discussion

In rodents, the β_3-adrenoceptor is the predominant β-adrenoceptor subtype in brown adipose tissue (Muzzin *et al.*, 1991). In newborn human brown adipose tissue the predominant β-adrenoceptor mRNA is, as in white adipose tissue (Mauriège *et al.*, 1988), that of the β_2-adrenoceptor and the β_3-adrenoceptor mRNA amounts to 9% of the total β-adrenoceptor mRNA.

The results of the binding study performed in human newborn brown adipose tissue plasma membranes, using [^{3}H]-CGP 12177, are very similar to those obtained in human omental fat membranes (Revelli *et al.*, 1993). In the latter study, two populations of binding sites with K_d values of 1.7 and 22 nM and B_{max} values of 124 and 402 fmol mg^{-1} of proteins, respectively, were identified (Revelli *et al.*, 1993). The [^{3}H]-CGP 12177 high affinity binding sites would represent the β_1/β_2-adrenoceptors and the low affinity binding sites the β_3-adrenoceptor (Revelli *et al.*, 1993). To test the latter hypothesis, binding studies with the new β_3-adrenoceptor agonist [^{3}H]-SB 206606, were performed. In human white adipose tissue membranes, where a large population of [^{3}H]-CGP 12177 low affinity binding sites was described, no [^{3}H]-SB 206606 binding could be detected. In human newborn brown adipose tissue plasma membranes comparison of the B_{max} values in Table 2 shows that the interaction of the [^{3}H]-SB 206606 with the β_3-adrenoceptor is only 35% of the low affinity [^{3}H]-CGP 12177 binding value. These results suggest that the low affinity [^{3}H]-CGP 12177 binding sites of human adipose tissue cannot be considered to consist only of β_3-adrenoceptors.

The K_d of [^{3}H]-SB 206606 binding to human newborn brown adipose tissue plasma membranes is similar to that of 38 nM reported in rat brown adipose tissue (Muzzin *et al.*, 1994). It is 17 fold lower than the K_i of BRL 37344 measured by Liggett (1992) in CHO cells transfected with the human β_3-adrenoceptor for the displacement of [^{125}I]-iodocyanopindolol. Thus, the species (rodent-human) differences in the affinity of

Table 2 Saturation kinetics of [^{3}H]-CGP 12177 and [^{3}H]-SB 206606 binding to human brown adipose tissue membranes

	[^{3}H]-CGP 12177	
	K_d(nM)	B_{max}(fmol mg^{-1})
High affinity sites	1.9 ± 0.6 (3)	156 ± 13 (3)
Low affinity sites	29 ± 12 (3)	483 ± 78 (3)
	[^{3}H]-SB 206606	
	K_d(nM)	B_{max}(fmol mg^{-1})
	87 ± 6 (4)	167 ± 25 (4)

The values are the mean ± s.e. of the number of experiments in parentheses. The inhibition affinity constants (K_i) are expressed in nM and the total number of sites (B_{max}) in fmol of ligand bound per mg of cell membrane proteins.

BRL 37344 for the β_3-adrenoceptor seems much less marked than previously claimed (Liggett, 1992) with transfected cells. The relatively low affinity of BRL 37344 for the β_3-adrenoceptor reported by Liggett (1992) might be due to the use of transfected cells or of [^{125}I]-iodocyanopindolol which is a relatively poor ligand to measure an interaction with the β_3-adrenoceptor. Surprisingly, despite the fact that the β_3-adrenoceptor mRNA represents only 9% of the total β-adrenoceptor mRNA in human brown adipose tissue, the density of β_3-adrenoceptors is rather high, i.e. 52% of the total β-adrenoceptors in this tissue and only 2.8 fold lower than that reported in rat brown adipose tissue (Muzzin *et al.*, 1994). The difference in the relative levels of the β_3-adrenoceptor mRNA and of the receptor protein might be due to a high turn-over rate of the mRNA as compared to that of the receptor protein. The biological importance of the β_3-adrenoceptor in human brown adipose tissue, in particular its coupling efficiency and its functional role in the lipolytic and thermogenic responses to catecholamines deserves further study.

It should be stressed that the use of [^{3}H]-SB 206606 to study the β_3-adrenoceptor in cell membranes has more limitations in human than in rat adipose tissue. In the rat the non-specific [^{3}H]-SB 206606 binding value in interscapular brown adipose tissue plasma membranes is 52% of the total binding value (Muzzin *et al.*, 1994), whereas in human newborn brown adi-

pose tissue plasma membranes it amounts to 85% of the total binding value. Furthermore, in human brown adipose tissue plasma membranes, contrary to what was observed in the rat (Muzzin *et al.*, 1994), the non-specific binding can be displaced non stereoselectively by high concentrations of BRL 37344. The specific binding value has therefore to be defined strictly as the part of the total binding which is displaced stereoselectively.

It has been shown in bovine perirenal tissue that the conversion of brown into white-adipose tissue is characterized by a dramatic decrease in the β_3-adrenoceptor mRNA expression after the age of 3 months (Casteilla *et al.*, 1994). The present study also shows that the adult white adipocyte is characterized by a low expression of the β_3-adrenoceptor. The results of this study suggest that the β-adrenoceptor subtype distribution pattern and its developmental change in human brown and white adipose tissues differ from what was reported in rodents. It must be kept in mind that a direct comparison of the same fat depot in newborn and adult human was not performed. The omental fat is indeed practically absent in newborn and therefore could not be studied. It is noteworthy that in a recent study Granneman (1995) could not detect by nuclease protection assay any β_3-adrenoceptor in infant omental white fat.

It has been reported that BRL 37344 has no or a very low lipolytic effect on human isolated white adipocytes (Langin *et al.*, 1991; Lönnqvist *et al.*, 1993). This is in agreement with the finding of the present study that the β_3-adrenoceptor is expressed in human white adipose tissue at a level which is insufficient to allow for its detection by Northern blot analysis of total RNA or by binding studies. On the other hand, a lipolytic effect of CGP 12177 has been demonstrated in human isolated white adipocytes (Lönnqvist *et al.*, 1993, 1995; Enocksson *et al.*, 1995). The present study demonstrates the existence of low affinity [^{3}H]-CGP 12177 binding sites in human omental fat membranes. It could be speculated that these sites are in fact those involved in the mediation of the lipolytic effects of CGP 12177. Further work is needed to support this hypothesis.

A significant level of expression of the β_3-adrenoceptor was demonstrated in human brown adipose tissue, suggesting that this receptor might play an important role in the biological response of this tissue to catecholamines.

We are greatly indebted to Prof. A. Rohner, to Prof. Claude Lecoultre, and to Dr S. Widgren and their groups at the Departments of Surgery, Paediatrics and Pathology of the Geneva Faculty of Medicine for their invaluable help in obtaining human fat tissue. This work was supported by a grant (No 32-33779.92) from the Swiss National Science Foundation and by a grant in aid from Sandoz-Stiftung zur Förderung der medizinisch-biologischen Wissenschaften.

References

ARCH, J.R.S. & KAUMANN, A.J. (1993). β_3 and atypical β-adrenoceptors. *Med. Res. Rev.*, **13**, 663–729.

BENSAID, M., KAGHAD, M., RODRIGUEZ, M., LE FUR, G. & CAPUT, D. (1993). The rat β_3-adrenergic receptor gene contains an intron. *FEBS Lett.*, **318**, 223–226.

CASTEILLA, L., MUZZIN, P., REVELLI, J.-P., RICQUIER, D. & GIACOBINO, J.-P. (1994). Expression of β_1- and β_3-adrenergic receptor messages and adenylate cyclase β-adrenergic response in bovine perirenal adipose tissue during its transformation from brown into white fat. *Biochem. J.*, **297**, 93–97.

CHUNG, F.Z., LENTES, U.K., GOCAYNE, J., FITZGERALD, M., ROBINSON, D., KERLAVAGE, A.R., FRASER, C.M. & VENTER, J.C. (1987). Cloning and sequence analysis of the human brain β-adrenergic receptor. Evolutionary relationship to rodent and avian β-receptors and porcine muscarinic receptors. *FEBS Lett.*, **211**, 200–206.

EMORINE, L.J., MARULLO, S., BRIEND-SUTREN, M.M., PATEY, G., TATE, K., DELAVIER-KLUTCHKO, C. & STROSBERG, A.D. (1989). Molecular characterization of the human β_3-adrenergic receptor. *Science*, **245**, 1118–1121.

ENOCKSSON, S., SHIMAZU, M., LÖNNQVIST, F., NORDENSTRÖM, J. & ARNER, P. (1995). Demonstration of an in vivo functional β_3-adrenoceptor in man. *J. Clin. Invest.*, **95**, 2239–2245.

FEVE, B., EMORINE, L.J., LASNIER, F., BLIN, N., BAUDE, B., NAHMIAS, C., STROSBERG, A.D. & PAIRAULT, J. (1991). Atypical β-adrenergic receptor in 3T3-F442A adipocytes: pharmacological and molecular relationship with the human β_3-adrenergic receptor. *J. Biol. Chem.*, **266**, 20329–20336.

GIACOBINO, J.-P. (1979). Subcellular fractionation of brown adipose tissue. *J. Supramol. Struct.*, **11**, 445–449.

GIACOBINO, J.-P. (1995). β_3-Adrenoceptor: an update. *Eur. J. Endocrinol.*, **132**, 377–385.

GRANNEMAN, J.G. (1995). Why do adipocytes make the β_3-adrenergic receptor? *Cell Signalling*, **7**, 9–15.

GRANNEMAN, J.G. & LAHNERS, K.N. (1992). Differential adrenergic regulation of β_1- and β_3-adrenoreceptor messenger ribonucleic acids in adipose tissues. *Endocrinology*, **130**, 109–114.

GRANNEMAN, J.G., LAHNERS, K.N. & CHAUDHRY, A. (1992). Molecular cloning and expression of the rat β_3-adrenergic receptor. *Mol. Pharmacol.*, **40**, 895–899.

GRANNEMAN, J.G., LAHNERS, K.N. & CHAUDHRY, A. (1993). A characterization of the human β_3-adrenergic receptor gene. *Mol. Pharmacol.*, **44**, 264–270.

HOLLENGA, CH., BROUWER, F. & ZAAGSMA, J. (1991). Relationship between lipolysis and cyclic AMP generation mediated by atypical β-adrenoceptors in rat adipocytes. *Br. J. Pharmacol.*, **102**, 577–580.

KRIEF, S., LÖNNQVIST, F., RAIMBAULT, S., BAUDE, B., VAN SPRONSEN, A., ARNER, P., STROSBERG, A.D., RICQUIER, D. & EMORINE, L.J. (1993). Tissue distribution of β_3-adrenergic receptor mRNA in man. *J. Clin. Invest.*, **91**, 344–349.

LAI, E., ROSEN, O.M. & RUBIN, C.S. (1982). Dexamethasone regulates the β_3-adrenergic receptor subtype expressed by 3T3-L1 preadipocytes and adipocytes. *J. Biol. Chem.*, **257**, 6691–6696.

LANGIN, D., PORTILLO, M.P., SAULNIER-BLACHE, J.-S. & LAFONTAN, M. (1991). Coexistence of three β-adrenoceptor subtypes in white fat cells of various mammalian species. *Eur. J. Pharmacol.*, **199**, 291–301.

LEHRACH, H., DIAMOND, D., WOZNEY, J.M. & BOEDTKER, H. (1977). RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. *Biochemistry*, **16**, 4743–4751.

LEVIN, B.E. & SULLIVAN, A.C. (1986). Beta-1 receptor is the predominant beta-adrenoreceptor on rat brown adipose tissue. *J. Pharmacol. Exp. Ther.*, **236**, 681–688.

LIGGETT, S.B. (1992). Functional properties of the rat and human β_3-adrenergic receptors: differential agonist activation of recombinant receptors in Chinese hamster ovary cells. *Mol. Pharmacol.*, **42**, 634–637.

LÖNNQVIST, F., KRIEF, S., STROSBERG, A.D., NYBERG, B., EMORINE, L.J. & ARNER, P. (1993). Evidence for a functional β_3-adrenoceptor in man. *Br. J. Pharmacol.*, **110**, 929–936.

LÖNNQVIST, F., THÖRNE, A., NILSELL, K., HOFFSTEDT, J. & ARNER, P. (1995). A pathogenic role of visceral fat β_3-adrenoceptors in obesity. *J. Clin. Invest.*, **95**, 1109–1116.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

MAURIEGE, P., DE PERGOLA, G., BERLAN M. & LAFONTAN, M. (1988). Human fat cell β-adrenergic receptors: β-agonist-dependent lipolytic responses and characterization of β-adrenergic binding sites on human fat cell membranes with highly selective β_1-antagonists. *J. Lip. Res.*, **29**, 587–601.

MUNSON, P.J. & RODBARD, D. (1980). LIGAND: a versatile computerized approach for the characterization of ligand binding systems. *Anal. Biochem.*, **107**, 220–239.

MUZZIN, P., COLOMB, C., GIACOBINO, J.-P., VENTER, J.C. & FRASER, C.M. (1988). Biochemical characterization of brown adipose tissue β-adrenergic receptor. *J. Receptor Res.*, **8,** 713–729.

MUZZIN, P., REVELLI, J.-P., KÜHNE, F., GOCAYNE, J.D., McCOMBIE, W.R., VENTER, J.C., GIACOBINO, J.-P. & FRASER, C.M. (1991). An adipose tissue-specific β-adrenergic receptor. Molecular cloning and down-regulation in obesity. *J. Biol. Chem.*, **266,** 24053–24058.

MUZZIN, P., REVELLI, J.-P., FRASER, C.M. & GIACOBINO, J.-P. (1992). Radioligand binding studies of the atypical β_3-adrenergic receptor in rat brown adipose tissue using [^{3}H]–CGP-12177. *FEBS Lett.*, **298,** 162–164.

MUZZIN, P., BOSS, O., MATHIS, N., REVELLI, J.-P., GIACOBINO, J.-P., WILLCOCKS, K., BADMAN, G.T., CANTELLO, B.C.C., HINDLEY, R.M. & CAWTHORNE, M.A. (1994). Characterization of a new, highly specific, β_3-adrenergic receptor radioligand, [^{3}H]SB 206606. *Mol. Pharmacol.*, **46,** 357–363.

NAHMIAS, C., BLIN, N., ELALOUF, J.-M., MATTEI, M.G., STROSBERG, A.D. & EMORINE, L.J. (1991). Molecular characterization of the mouse β_3-adrenergic receptor: relationship with the atypical receptor of adipocytes. *EMBO J.*, **10,** 3721–3727.

OKAYAMA, H., KAWAICHI, M., BROWNSTEIN, M., LEE, F., YOKOTA, T. & ATAI, K. (1987). High-efficiency cloning of full-length cDNA; construction and screening of cDNA expression libraries for mammalian cells. In *Methods in Enzymology*, eds Wu, R. & Crossman, L., Vol. 154, pp. 3–28. Orlando, New York, San Francisco, London: Academic Press Inc.

REVELLI, J.-P., MUZZIN, P., PAOLONI, A., MOINAT, M. & GIACOBINO, J.-P. (1993). Expression of the β_3-adrenergic receptor in human white adipose tissue. *J. Mol. Endocrinol.*, **10,** 193–197.

RODBELL, M. (1964). Metabolism of isolated fat cells. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.*, **239,** 375–380.

ROTHWELL, N.J., STOCK, M.J. & SUDERA, D.K. (1985). β-Adrenoceptors in rat brown adipose tissue: proportions of β_1- and β_2-subtypes. *Am. J. Physiol.*, **248,** E397–E402.

(Received February 5, 1996
Accepted March 5, 1996)