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Lipolytic effects of conventional β_3-adrenoceptor agonists and of CGP 12,177 in rat and human fat cells : preliminary pharmacological evidence for a putative β_4-adrenoceptor

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1 The nature of rat and human fat cell β_3-adrenoceptors was investigated by studying the effects of the new β_3-adrenoceptor selective antagonist, SR 59,230A, on lipolysis induced by the conventional β_3-adrenoceptor agonists, CL 316,243 and SR 58,611A, and by the non-conventional partial β_3-adrenoceptor agonist CGP 12,177 (a potent β_1- and β_2-adrenoceptor antagonist with partial β_3-adrenoceptor agonist property).

2 In rat fat cells, the rank order of potency of agonists was: CL 316,243 > isoprenaline > SR 58,611A > CGP 12,177. The three former agents were full agonists whereas CGP 12,177 was a partial agonist (intrinsic activity of 0.70). In human fat cells, the lipolytic effect of CGP 12,177 reached 25 % of isoprenaline effect. CL 316,243 was a poor inducer of lipolysis and SR 58,611A was ineffective.

3 In rat fat cells, lipolysis induced by CL 316,243 and SR 58,611A was competitively antagonized by SR 59,230A. Schild plots were linear with pA_2 values of 6.89 and 6.37, respectively. Conversely, 0.1, 0.5 and 1 μM SR 59,230A did not modify the concentration-response curve of CGP 12,177. A rightward shift of the curve was however observed with 10 and 100 μM of SR 59,230A. The apparent pA_2 value was 5.65. The non-selective β-adrenergic antagonist, bupranolol, competitively displaced the concentration-response curve of CGP 12,177 and CL 316,243. Schild plots were linear with pA_2 values of 6.70 and 7.59, respectively. CL316,243-mediated lipolytic effect was not antagonized by CGP 20,712A. In human fat cells, CGP 12,177-mediated lipolytic effect was antagonized by bupranolol and CGP 20,712A. SR 59,230A (0.1, 1 and 10 μM) did not modify the concentration-response curve of CGP 12,177. A rightward shift was however observed at 100 μM leading to an apparent pA_2 value of 4.32.

4 The results suggest that the non-conventional partial agonist CGP 12,177 can activate lipolysis in fat cells through the interaction with a β-adrenoceptor pharmacologically distinct from the β_3-adrenoceptor, i.e. through a putative β_4-adrenoceptor. They suggest that the two subtypes coexist in rat fat cells whereas only the putative β_4-adrenoceptor mediates lipolytic effect of CGP12,177 in human fat cells.

Keywords: Lipolysis; human adipocytes; CL 316,243; SR 58,611A; SR 59,230A; CGP 20712A; bupranolol; white adipose tissue

Introduction

The lipolytic effects generated by catecholamines in white fat cells was initially defined in terms of β_1- and/or β_2-adrenoceptor (AR)-mediated activation. The presence of β_1- and β_2-ARs has been clearly established in human (Burns *et al*., 1981; Mauriège *et al*., 1988; Lafontan & Berlan, 1993) and non human primate (Bousquet-Mélou *et al*., 1994; 1995) fat cells. The lipolytic responses and binding assays were well correlated (Mauriège *et al*., 1988) and in agreement with the standard classification of Lands *et al*. (1967). In rodents, the main lipolytic effect of catecholamines was attributed to the activation of a third β-AR with a small subordinate role of the β_1-ARs (Wilson *et al*., 1984; Bojanic *et al*., 1985; Hollenga & Zaagsma, 1989; Langin *et al*., 1991; Collins *et al*., 1994). The presence of a β_3-AR in brown and white fat cells was established by the pharmacological analysis of the β-AR-mediated metabolic responses (i.e. oxygen consumption, lipolysis, adenylyl cyclase activity and adenosine 3′:5′-cyclic monophosphate (cyclic AMP) accumulation) (Lafontan & Berlan, 1993). β_3-ARs have also been described in the gastric fundus, the jejunum and the colon of the rat and the rabbit (Norman & Leathard, 1990; McLaughlin & Donald, 1991; Manara *et al*., 1995). Evidence for a β_3-AR was further sustained by the isolation of the β_3-AR gene in various species (Emorine *et al*., 1989; Nahmias *et al*., 1991; Pietri-Rouxel *et al*., 1995; Atgié *et al*., 1996). In transfected CHO cells, the product of the gene was shown to activate adenylyl cyclase with pharmacological properties similar to those defined in rat fat cells. β_3-AR agonists such as BRL 37,344, CL 316,243, SR 58611A and CGP 12,177 potently stimulate lipolysis in rodent brown and white fat cells (Bojanic *et al*., 1985; Hollenga & Zaagsma, 1989; Langin *et al*., 1992; Van Liefde *et al*., 1992; Himms Hagen *et al*., 1994) and activate cyclicAMP accumulation in β_3-AR expressing CHO cells (Strosberg & Pietri-Rouxel, 1996).

The existence and role of β_3-ARs in human adipocytes is a much debated question. High concentrations of BRL 37,344 and CL 316,243 are necessary to promote lipolysis. The effect of these agonists is antagonized by β_1- and β_2-AR antagonists suggesting a loss of selectivity (Hoffstedt *et al*., 1996; Tavernier *et al*., 1996; Umekawa *et al*., 1996). SR 58,611 does not show any lipolytic effect (Tavernier *et al*., 1996). Similar results have been obtained in non-human primates (Bousquet-Mélou *et al*., 1994). Moreover, CL 316,243, which shows a higher selectivity than BRL 37,344 on the rodent β_3-AR, does not induce lipolysis *in vivo* in baboons (Shen *et al*., 1996). Among the β_3-AR agonists, CGP 12,177 showed the highest efficacy in human fat cells (Barbe *et al*., 1996; Hoffstedt *et al*., 1996; Tavernier *et al*., 1996; Umekawa *et al*., 1996). Since CGP 12,177 is a β_1- and β_2-AR antagonist, its lipolytic effect is not mediated by β_1- and β_2-ARs and was thus attributed by most authors to β_3-AR. Determination of CGP 12,177-induced lipolytic effect was there-

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fore used as an index of variations in β_3-AR-mediated lipolysis in man (Lönnqvist *et al*., 1993, 1995).

Several hypotheses have been proposed to explain the discrepancy between primate and rodent fat cells including structural differences of β_3-ARs in various species and differences in the expression level of the receptor (Arch & Kaumann, 1993; Strosberg & Pietri-Rouxel, 1996). The pharmacological profile of the putative β_3-AR present on human and non-human primates fat cells does not fulfill the four criteria for an effect mediated by β_3-ARs (Arch & Kaumann, 1993; Kaumann & Molenaar, 1996). The first criterion (high potency for selective β_3-AR agonists) is not achieved in human fat cells. The second and third criteria are partially fulfilled, i.e. stimulation by non-conventional partial agonists and resistance to blockade by β_1- and β_2-AR antagonists, respectively (Tavernier *et al*., 1996). The fourth criterion (blockade of the receptor by selective β_3-AR antagonists) has never been evaluated in human adipose tissue.

Since CGP 12,177 has been used as the reference β_3-AR agonist in human fat cells, the main goal of the present study was to determine if the lipolytic effect of CGP 12,177 was mediated by β_3-ARs. CGP 12,177 and conventional β_3-AR agonist effects were studied in the presence and absence of the non-selective β-AR antagonist, bupranolol (Langin *et al*., 1991; Galitzky *et al*., 1993; Tavernier *et al*., 1996), the selective β_1-AR antagonist, CGP 20,712A and the new selective β_3-AR antagonist, SR 59,230A (Manara *et al*., 1996; Nisoli *et al*., 1996). The results provide preliminary pharmacological evidence for the presence of a putative β_4-AR on fat cells.

Methods

Adipose tissue

Rat white adipocytes were prepared from the epididymal fat pads of 30 male Wistar rats (180–230g). Human subcutaneous adipose tissue was removed from 14 non-obese or moderately obese women undergoing plastic surgery. Their mean age $\pm$s.d. was 39.5$\pm$ 10.4 years and their mean body mass index (weight/height^2) was 26.8$\pm$3.3 kg/m^2. Samples of subcutaneous abdominal adipose tissue (0.5–1 g) were taken in the morning at the beginning of the operation. The study was approved by the Ethical Committee of Toulouse University Hospital.

Adipocyte preparation and lipolysis measurements

Isolated adipocytes were obtained as previously described (Tavernier *et al*., 1996) by collagenase digestion of adipose fragments in Krebs-Ringer bicarbonate buffer containing albumin (2 g 100 ml^{-1}) (KRBA) and glucose (6 mM) at pH 7.4 and 37°C under gentle shaking at around 60 cycles · min^{-1}. At the end of the incubation, fat cells were filtered through a silk screen and washed three times with KRBA buffer to eliminate collagenase. Packed cells were brought to a suitable dilution in KRBA buffer for lipolysis. Pharmacological agents at suitable dilutions were added to the cell suspension (2000–3000 cells/assay) just before the beginning of the assay in a final volume of 100 μl. After 90 min of incubation, the tubes were placed in an ice bath and 20 to 50 μl aliquots of the infranatant were taken for enzymatic determination of the glycerol (Bradley & Kaslow, 1989) released in the incubation medium which was used as the index of fat cell lipolysis.

The pharmacological agents used to study β-adrenoceptor activation of lipolysis were the non-selective β-AR agonist, isoprenaline, and three β_3-AR-mediated lipolytic drugs defined on rat and dog (Bousquet-Mélou *et al*., 1994) fat cells: CGP 12,177 (initially defined as a β_1-/β_2-AR antagonist), SR 58,611A and CL 316,243. The selective β_3-AR antagonist, SR 59,230A, the selective β_1-AR antagonist, CGP 20,712A and the non-selective β-AR antagonist, bupranolol, were used.

Chemicals

GP 12,177 (4-[3-t-butylamino-2-hydroxypropoxy]benzimidazol-2-one) and CGP 20,712A ((±)-[2-(3-carbomyl-4-hydroxyphenoxy) - ethylamino] - 3-[4-1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxy]2-propanol methane sulphonate) were generous gifts from Ciba Geigy (Basle, Switzerland). CL 316,243, ((**R**,**R**) -5- [2-[[2-(3- chlorophenyl) - 2- hydroxyethyl]-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate) was from the Medical Research Division, Lederle Laboratories, American Cyanamid Company (Pearl River, New York, U.S.A). SR 58,611A (N[2**S**)-7-carbethoxymethoxy-1,2,3,4-tetrahydronaphth-2-yl]-(2**R**)-2-hydroxy-2-chlorophenyl) ethanamine hydrochloride) and SR 59,230A ((3-(2-ethylphenoxy)-1[(1**S**)-1,2,3,4-tetrahydronaphth - 1-ylaminol] - (2**S**)2-propanol- oxalate) were kindly provided by Dr. L. Manara (Sanofi-Midy group, Milano, Italy). (±)±-Bupranolol (1-(2-choro-5-methylphenoxy)-3-[(1,1-dimethylethyl)amino]-2-propanol) was from Schwarz Pharma (Monheim, Germany). Bovine serum albumin (fraction V) was from Sigma (Paris, France). Crude collagenase and other enzymes came from Boehringer Mannheim (Mannheim, Germany).

Data analysis

Values are given as mean $\pm$s.e.mean of 6 to 8 separate experiments. Student's paired *t*-test was used for comparisons between matched pairs. Differences were considered significant when P was smaller than 0.05. The intrinsic activity (IA) of each drug was calculated by dividing its maximal lipolytic effect by the maximal effect of the full agonist isoprenaline. Half-maximal effective drug concentration (EC_{50}) values were obtained by computer fitting of concentration-response curves of various β-AR agonists obtained in the presence or in the absence of various concentrations of antagonist. Schild plots were constructed according to Arunlakshana & Schild (Arunlakshana & Schild, 1959) and their slopes were determined by linear regression analysis. When the slope did not significantly differ from unity, pA_2 values were calculated for each concentration of antagonist according to the equation $pA_2 = \log([\text{antagonist}]/(DR-1))$.

Results

Figure 1 shows a comparison of the lipolytic effects of isoprenaline, CL 316,243, SR 58,611A and CGP 12,177. Spontaneous glycerol release (basal lipolysis) was 0.29$\pm$0.06 and 0.32$\pm$ 0.08 μmol/100 mg lipid for human and rat fat cells, respectively. Maximum lipolytic effects of isoprenaline were 1.18$\pm$0.26 and 2.25 $\pm$ 0.28 μmol 100 mg^{-1} lipid for human and rat fat cells, respectively. The pD_2 values and intrinsic activities (IA) are shown in Table 1. In rat fat cells, CL 316,243 and SR 58,611A showed IA which were not different from 1, whereas in human fat cells, CL 316,243 showed a very low IA (a significant increase in glycerol production was only measured with 100 μM). In human fat cells, SR 58,611A was not lipolytic. In rat and human fat cells, CGP 12,177 was a partial agonist since IA (0.70$\pm$0.05 and 0.24$\pm$5.2, respectively) were significantly lower than 1. In rat fat cells, the relative rank order of potency was: CL 316,243 > isoprenaline > SR 58,611A > CGP 12,177. The rank order of potency differed in human fat cells (isoprenaline >> CGP 12,177 >> CL 316,243).

Figure 2a shows, on rat fat cells, that 100 μM bupranolol and SR 59,230A completely antagonized the lipolytic effect induced by 0.01 μM CL 316,243. CGP 20,712A (100 μM) inhibited 23% of the CL 316,243 effect. The inhibitory effects of the antagonists were studied on 10 μM CGP 12,177-mediated lipolytic effects in rat and human fat cells. In rat fat cells, 100 μM SR 59,230A and CGP 20,712A inhibited 64 and 50% of the CGP 12,177-induced effect, respectively (Figure 2b). Bupranolol (100 μM) completely suppressed CGP 12,177-induced lipolysis. In human fat cells, CGP 20,712A and bupra-

nolol completely antagonized the lipolytic effect induced by 10 μM CGP 12,177. SR 59,230A was ineffective (Figure 2c).

Figure 3 shows the concentration-response curves of CL 316,243 and SR 58,611A in the presence of increasing concentrations of SR 59,230A on rat fat cells. For both agonists, a clear rightward shift was observed. Schild plots were linear with slopes not different from unity (0.88 and 1.03 for CL 316,243 and SR 58,611A, respectively). The pA_2 calculated were 6.89 and 6.37 for SR 59,230A vs responses to CL 316,243 and SR 58,611A, respectively. These results suggest that SR 59,230A acts as a specific antagonist of the lipolytic effects induced by the β_3-AR agonists, CL 316,243 and SR 58,611A.

The subtype selectivity of the CGP 12,177 effect was measured, by use of SR 59,230A, on rat and human fat cells. Figure 4 shows that, at low concentrations of SR 59,230A (0.1 to 1 μM), no significant rightward shift of the CGP 12,177 concentration-response curve was found on rat fat cells. The rightward shift appeared when higher concentrations of antagonist (10 and 100 μM) were applied. In consequence, the calculated logarithm of the dose-ratio (log DR-1) was not modified at the three lowest concentrations of antagonist whereas it increased at the two highest. The Schild plot from these data were clearly biphasic, the slope of the steep part (1, 10 and 100 μM SR 59,230A) being not significantly different from unity (1.01). From these points, an apparent pA_2 of 5.65 was calculated (Hollenga *et al*., 1991). On human fat cells, no shift of CGP 12,177 concentration-response curve was observed with concentrations up to 10 μM of SR 59,230A. A significant rightward shift was only observed at 100 μM. The Schild plot constructed from the data clearly indicated that SR 59,230A did not act as an antagonist towards the lipolytic

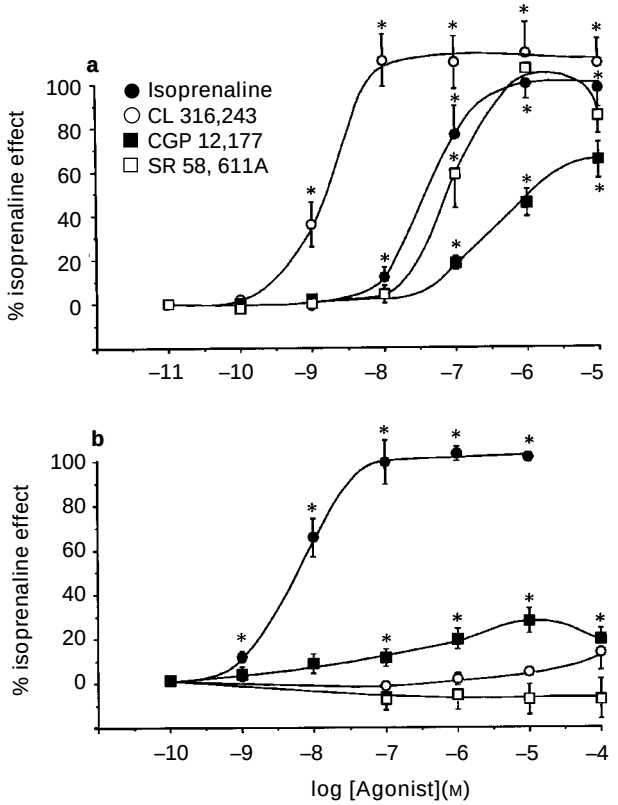

Figure 1 Comparison of the lipolytic effects of isoprenaline, CL 316,243 CGP, 12,177 and SR 58,611A in rat (a) or human (b) fat cells. Lipolysis is expressed in percentage of the maximal response obtained with isoprenaline. Values are the mean of 6 experiments (rat) or 8 experiments (human); vertical lines show s.e.mean. *Significantly different from spontaneous glycerol release ($P<0.05$).

Table 1 Comparative lipolytic effects of isoprenaline, CL 316,243, SR 58,611A and CGP 12,177 on rat and human fat cells

	IA		*pD₂*	
	Rat	*Human*	*Rat*	*Human*
Isoprenaline	1	1	7.44±0.12	8.07±0.4
CL 316,243	1.05±0.05	0.12±0.06*	8.88±0.11	ND
SR 58,611A	0.96±0.05	0	7.06±0.06	–
CGP 12,177	0.70±0.05*	0.24±0.05*	6.43±0.11	6.23±0.07

Data shown are means±s.e.means ($n=6$ (rat) and 8 (human)) IA: intrinsic activity (maximum effect of selective agents with respect to the maximum effect of isoprenaline). $pD_2 = -\log EC_{50}$ (EC_{50}: concentration of agonist inducing half-maximum effect). ND: not determined. *Significantly different from the maximal lipolytic effect of isoprenaline ($P<0.05$).

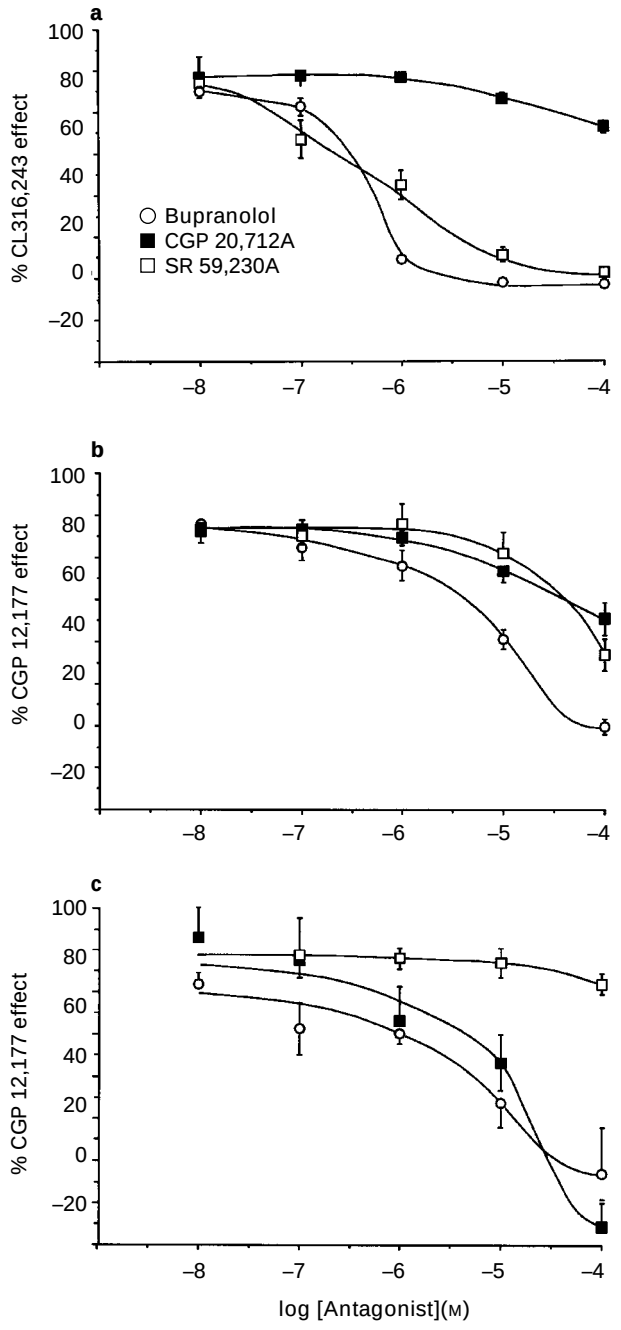

Figure 2 Antagonism of 0.01 μM CL 316,243- and 10 μM CGP 12,177-induced lipolysis by increasing concentrations of bupranolol, CGP 20,712A and SR 59,230A in rat (a, b) and human (c) fat cells. Lipolysis is expressed as percentage of the response obtained with the indicated concentrations of CL 316,243 and CGP 12,177. Values are the mean of 6 experiments; vertical lines show s.e.mean.

effect of CGP 12,177 at concentrations up to 10 μM. The apparent pA_2 value calculated from 10 and 100 μM SR 59,230A was 4.32.

Bupranolol, a non-selective β-AR antagonist, has been demonstrated to block the effect of CGP 12,177 on fat cells from animal models (Langin *et al*., 1991; Galitzky *et al*., 1993) and man (Tavernier *et al*., 1996). In order to assess whether bupranolol was able to antagonize the effects of CGP 12,177, further experiments were performed on rat fat cells (Figure 5). The concentration-response curves of CGP 12,177 and CL 316,243 were competitively displaced by increasing concentrations of bupranolol. Schild plots were linear with slopes not different from unity (0.8 and 1.19 for CGP 12,177 and CL 316,243, respectively). Calculated pA_2 values were 6.70 and 7.59, respectively.

Discussion

The comparison of the lipolytic effects of isoprenaline with those of the three β_3-AR agonists in rat and human fat cells is in agreement with previous data (Bousquet-Mélou *et al*., 1994; Hoffstedt *et al*., 1996; Tavernier *et al*., 1996; Umekawa *et al*., 1996). The non-conventional partial β_3-AR agonist, CGP 12,177 (defined as a β_1- and β_2-AR antagonist with partial agonist activity at high concentrations in rodents), was the only drug able to activate lipolysis in human. Conventional β_3-AR selective agonists such as CL 316,243 and SR 58,611A failed to induce β_3-AR specific lipolytic effects on human fat cells but acted as full lipolytic agents on rat fat cells (Figure 1 and Table 1). It has been shown that the partial lipolytic action of BRL 37,344 (Hollenga *et al*., 1990; Lönnqvist *et al*., 1993) and CL 316,243 occurring at high concentrations of agonists depends mainly on residual β_1- and β_2-ARs activities (Tavernier *et al*., 1996, Hoffstedt *et al*., 1996; Umekawa *et al*., 1996). Similar results have been obtained on fat cells from non-human primates (baboon and macaque) (Bousquet-Mélou *et al*.,

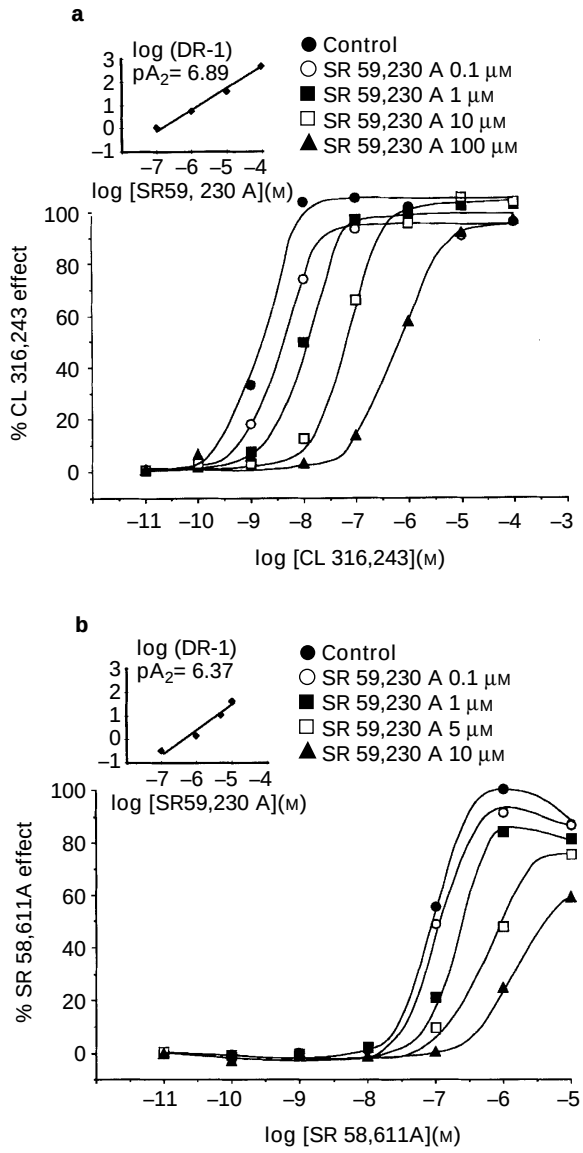

Figure 3 Antagonism of (a) CL 316,243- and (b) SR 58,611A-induced lipolysis by the selective β_3-adrenoceptor antagonist SR 59,230A on rat fat cells. Lipolysis is expressed in percentage of the maximum response obtained with CL 316,243 or SR 58,611A. Values are the mean of 6 experiments. Standard error was below 10% and were omitted for clarity. The insets show the corresponding Schild plots derived from the mean curves.

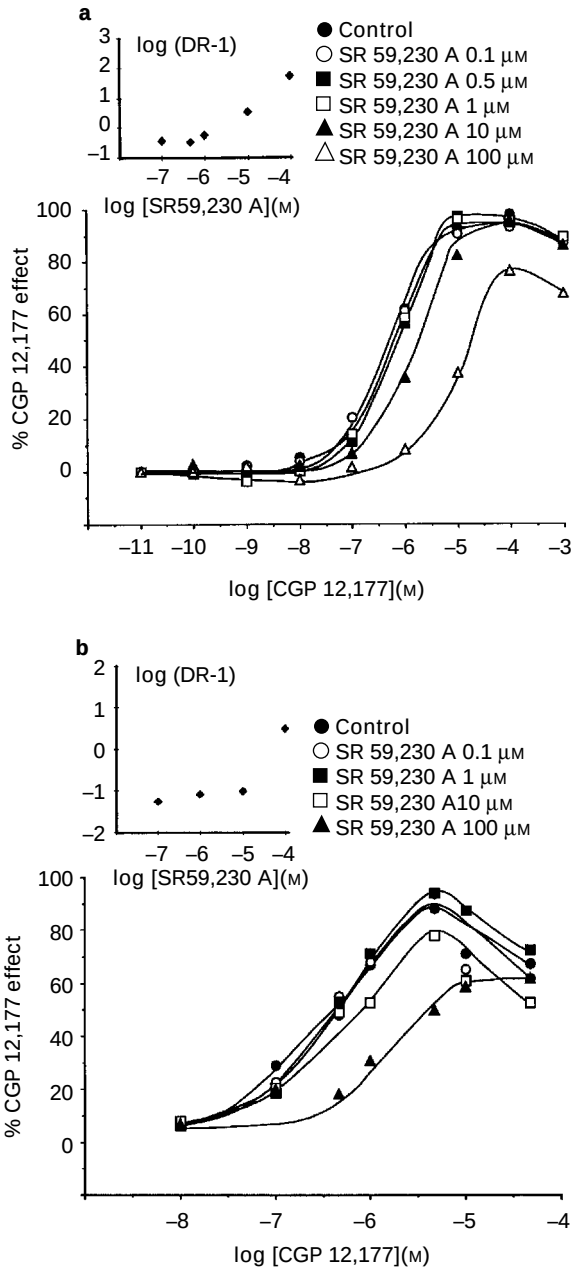

Figure 4 Antagonism of CGP 12,177-induced lipolysis by the selective β_3-adrenoceptor antagonist SR 59,230A in rat (a) or human (b) fat cells. Lipolysis is expressed in percentage of the maximal response obtained with CGP 12,177. Values are the mean of 6 experiments (rat) or 8 experiments (human). Standard error was below 10% and were omitted for clarity. The insets show the corresponding Schild plots derived from the mean curves.

1994). In these species, CGP 12,177 induced, similarly to human fat cells, a partial lipolytic effect (6 to 30% of isoprenaline effect), and other selective β_3-AR agonists were shown to be inactive.

The debate on β_3-AR-mediated biological effects is further complicated by the pharmacological differences observed between the human and rat β_3-AR. In CHO cells expressing β_3-ARs, BRL 37,344 and CL 316,243 are less potent on the human than the rat receptors (Strosberg & Pietri-Rouxel, 1996). The new β_3-AR antagonist SR 59,230A was shown to be as potent on the human and rat receptors (Levasseur *et al*., 1995). In CHO cells expressing human and rat β_3-ARs, the classical

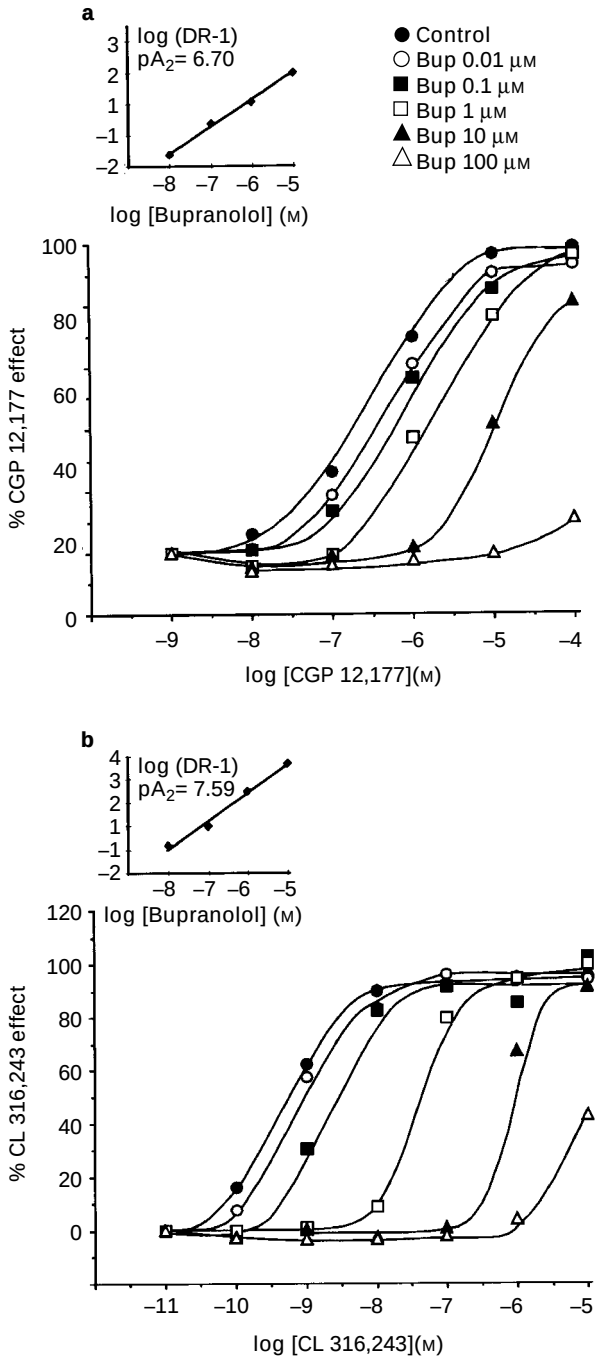

Figure 5 Antagonism of (a) CGP 12,177- and (b) CL 316,243-induced lipolysis by the non-selective β-adrenoceptor antagonist bupranolol (Bup) on rat fat cells. Lipolysis is expressed in percentage of the maximal response obtained with CGP 12,177 and CL 316,243. Values are the mean of 6 experiments. Standard error was below 10% and were omitted for clarity. The insets show the corresponding Schild plots derived from the mean curves.

β_3-AR agonists (i.e. BRL 37,344, CL 316,243 and SR 58,611A) showed higher intrinsic activities than CGP 12,177 that behaves as a partial agonist when measuring cyclic AMP accumulation (Strosberg & Pietri-Rouxel, 1996; Wilson *et al*., 1996). This pharmacological profile is in accordance with the one observed in rat fat cells but markedly different from the data obtained in human fat cells (Figure 1). However, with adenylyl cyclase assays on membranes from CHO cells transfected with the human β_3-AR gene, an opposite rank order of potency of β_3-AR agonists was described (i.e. CGP 12,177 > BRL 37,344); the efficacy of BRL 37,344 was very poor (Wilson *et al*., 1996). It is of note that BRL 37,344 shows an anomalous behaviour in the adenylyl cyclase assay (low intrinsic activity and low potency) performed on rat fat cell membranes (Hollenga *et al*., 1991) or CHO cell membranes (Wilson *et al*., 1996). However, measurement of cyclic AMP-dependent protein kinase activity ratio, gives a faithfull reflection of changes in cellular cyclic AMP levels, BRL 37,344 shows high potency and an intrinsic activity of 1 in the two systems (Langin *et al*., 1992; Wilson *et al*., 1996). Moreover, it is well known that there is a strong shift to the right of the agonist concentration-reponse curves (when compared with lipolysis or cyclic AMP accumulation) when the adenylyl cyclase assayis performed. Comparisons of drug efficacies and potencies in transfected systems must be interpreted with caution, since it has been shown that the level of expression of β_3-ARs has a major influence on the parameters obtained for the agonists (Wilson *et al*., 1996). Moreover, values obtained for agonist efficacies and potencies in transfected and native cells differ when assays based on intact cells are used (lipolysis, cyclic AMP accumulation and cyclic AMP-dependent protein kinase activation) and membranes (adenylyl cyclase activity).

Taken together, these intringuing data could suggest that the so-called β_3-AR expressed in human adipocytes is different from that present in rat fat cells. This led us to perform a complete characterization of the effects of the selective β_3-AR agonists and of the non-conventional partial agonist CGP 12,177 on both rat and human fat cells. Such a study became feasible by the use of SR 59,230A, an antagonist which has been shown to be more selective for β_3-AR than the non selective β-AR antagonists (Manara *et al*., 1996; Nisoli *et al*., 1996). In rat fat cells (Figure 2a), the lipolytic effect of CL 316,243 was completely antagonized by bupranolol and SR 59,230A but resistant to CGP 20,712A, a β_1-AR antagonist with very low affinity for the β_2-ARs and β_3-ARs (Strosberg & Pietri-Rouxel, 1996; Levasseur *et al*., 1997). This is consistent with a β_3-AR effect. Blockade of the effect of CGP 12,177 was different. SR 59,230A and CGP 20,712A only partially inhibited CGP 12,177-induced lipolysis whereas bupranolol completely blocked the effect (Figure 2b). These data suggest that CGP 12,177 interacts with the β_3-AR and with another receptor. In human fat cells, SR 59,230A did not inhibit CGP 12,177-induced lipolysis. The lipolytic effect was completely antagonized by bupranolol and CGP 20,712A (Figure 2c). This suggests that CGP 12,177 promotes lipolysis through a receptor pharmacologically distinct from a β_3-AR.

To characterize further this receptor, comparative studies with SR 59,230A were undertaken in rat and human fat cells. A clear competitive antagonism was observed, in rat fat cells, between SR 59,230A and CL 316,243 or SR 58,611A-induced lipolytic effects (Figure 3), showing that both drugs acted through the same receptor, i.e. the β_3-AR. Similar data have been obtained in various rat tissues by others with SR 59,230A (Manara *et al*., 1996; Nisoli *et al*., 1996; Levasseur *et al*., 1997). In sharp contrast, competition studies on CGP 12,177 effects in rat fat cells showed a non-linear Schild plot, since elevated concentrations of SR 59,230A were necessary to antagonize CGP 12,177-induced lipolysis. Because CGP 12,177 inhibition onset was only observed at very high concentrations of SR 59,230A, it can be concluded that the population of receptor which binds CGP 12,177 differs in part from that which binds CL 316,243 and SR 58,611A. In human fat cells, SR 59,230A was unable to displace the concentration-response curve of

CGP 12,177 until 10 μM. Again, this pharmacological behavior confirms that CGP 12,177 binds to a receptor which differs from the β_3-AR. The observation that bupranolol competitively displaced the lipolytic effect of CGP 12,177 in rat fat cells with a linear Schild plot (Figure 5) suggests that, unlike SR 59,230A, bupranolol is an antagonist of the two receptors recognized by CGP 12,177. This was also shown recently in human fat cells where bupranolol exhibited competitive antagonism towards the lipolytic effect of CGP 12,177 (Hoffstedt *et al*., 1996). Taken together, data obtained with bupranolol, CGP 20,712A and SR 59,230A suggest that CGP 12,177 binds to a novel β-AR, i.e. a putative β_4-AR.

This conclusion is also supported by studies on the level of expression of β_3-mRNAs. In rodent fat cells, β_3-AR mRNAs represent 90% of total β-AR transcripts (Collins *et al*., 1994). By contrast, in human subcutaneous fat cells, β_1- and β_2-AR mRNAs are readily detectable (Arner *et al*., 1990) and we showed recently that β_3-AR mRNAs represent less than 20% of total β-AR transcripts (Tavernier *et al*., 1996). Others found a low amount or no β_3-AR mRNAs in human adipose tissue (Krief *et al*., 1993; Revelli *et al*., 1993; Berkowitz *et al*., 1995; Deng *et al*., 1996).

The pharmacological profile of the putative β_4-AR present on human fat cells is close to that described in other biological systems. The existence of a β-AR different from the β_1-, β_2- and β_3-ARs has been suggested in different species and tissues, such as in rat smooth muscle (Oriowo, 1995), in rat stomach fundus (Cohen *et al*., 1995) and in guinea-pig taenia caecum (Koike *et al*., 1996). Such a receptor has also been described in human right atrium and in rat heart (Kaumann, 1996; Kaumann & Molenaar, 1996; Malinowska & Schlicker, 1996). In these tissues, CGP 12,177 promotes a positive inotropic effect which other agonists (CL 316,243, SR 58,611A and BRL 37,344) failed to show. This effect is moderately blocked by bupranolol and CGP 20,712A and resistant to SR 59,230A and propranolol blockade (Kaumann & Molenaar, 1996). These observations are in agreement with the present results since the lipolytic effects of CGP 12,177 were better antagonized by bupranolol and CGP 20,712A than by SR 59,230A. As demonstrated in human atrium (Kaumann, 1996; Kaumann & Molenaar, 1996), it is expected that the putative β_4-ARs present on fat cells cause an increase in cAMP level. Interestingly, it has been demonstrated that, on human ventricular endomyocardium, BRL 37,344, SR 58,611A, CL 316,243 and CGP 12,177 caused a negative inotropic effect through β_3-AR stimulation (Gauthier *et al*., 1996). These effects were markedly reduced after pretreatment with pertussis toxin, suggesting the involvement of Gi proteins in the β_3-AR signalling pathway of this tissue. In fact, previous studies have shown that in rat fat cells, unlike β_1-AR, β_3-AR could be coupled to both Gs and Gi proteins (Chaudhry *et al*., 1994). However, studies are necessary to elucidate the coupling of β_3- and β_4-ARs in fat cells.

Most importantly, the presence of a putative β_4-ARs in human fat cells reactivates the debate concerning the role of β_3-ARs in man and the use of β_3-AR agonists as therapeutic agents. Lönnqvist *et al*. (1995) have suggested that the increased lipomobilization from visceral human fat cells observed in obese patients was due to an elevated β_3-AR lipolytic effect. This conclusion was reached when the CGP 12,177-induced lipolytic effect was used as an index of β_3-AR-mediated lipolysis. Additionally, β_3-AR agonists were proposed as antiobesity drugs because of their ability to increase energy expenditure and improve glucose tolerance in rodents (Arch & Kaumann, 1993). The demonstration that none of the β_3-AR agonists stimulate lipolysis through β_3-ARs in human fat cells strongly suggests the need of a reassessment of the use β_3-AR agonists as potential antiobesity drugs.

In conclusion, the present data show that the non-conventional partial agonist CGP 12,177 can stimulate lipolysis in fat cells through the stimulation of a β-AR pharmacologically distinct from the β_3-AR, i.e. a putative β_4-AR. They suggest that the two subtypes coexist in rat fat cells, whereas only the β_4-AR mediates the CGP 12,177-induced lipolytic effects in human fat cells. Our data provide an explanation for the discrepancies often described in the lipolytic effect of conventional β_3-AR agonists in rodent and human fat cells and to the so far disappointing clinical trials with conventional β_3-AR agonists.

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