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Characterization of β_1- and β_3-adrenoceptors in intact brown adipocytes of the rat

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1 The binding properties of β_1-, β_2- and β_3-adrenoceptors were determined in isolated brown adipocytes of the rat rather than in membrane preparations from tissue homogenates, because typical brown adipocytes represent only about 40% of the various cells present in brown adipose tissue. Binding characteristics were assessed with the hydrophilic β-adrenoceptor radioligand, (−)-[^{3}H]-CGP 12177. The potent β-antagonist, bupranolol (100 μM) was used to determine nonspecific binding. Characterization was essentially performed by saturation and competition studies.

2 The saturation curve of (−)-[^{3}H]-CGP 12177 was clearly biphasic (Hill coefficient, nH = 0.57 ± 0.11, $P<0.01$) indicating the presence of two different β-adrenoceptor populations of high (K_D = 0.24 ± 0.04 nM) and low (K_D = 80 ± 7 nM) affinity. The low affinity sites were more numerous (B_{max} = 121 000 ± 30 000 sites/cell) than the high affinity sites (B_{max} = 12 000 ± 1 000 sites/cell).

3 (−)-[^{3}H]-CGP 12177 (25 nM) was displaced by adrenaline (Ad), noradrenaline (NA), isoprenaline (Iso), phenylephrine (Phe) and by the new β_3 agonist, CL 316 243 (CL) in a biphasic pattern. The order of potency for (−)-[^{3}H]-CGP 12177 displacement from the small population of high affinity sites (Iso ≫ NA > Ad ≫ CL ≫ Phe was in agreement with a β_1/β_2-classification. In contrast, the potencies of the same agonists for displacing the radioligand from the low affinity binding sites (CL ≫ Iso > NA > Ad ≫ Phe) revealed the presence of a distinct population of adrenoceptors obeying a β_3-classification. 5-HT did not displace (−)-[^{3}H]-CGP 12177 (25 nM) when used at concentrations as high as 0.1 mM.

4 The β-adrenoceptor antagonist, (−)-bupranolol, was more effective than (−)-propranolol for displacing (−)-[^{3}H]-CGP 12177 (25 nM) from the high (K_i = 0.029 ± 0.011 and 0.19 ± 0.07 nM, respectively and low (K_i = 0.27 ± 0.04 μM and 1.6 ± 0.2 μM, respectively) affinity binding sites. The selective β_1-antagonist CGP 20712A efficiently displaced the radioligand from a small population (K_i = 65 ± 19 pM) of binding sites, confirming the presence of β_1-adrenoceptors.

5 To evaluate whether β_2-adrenoceptors could be identified in the population of high affinity binding sites, displacement studies were performed at a low concentration of (−)-[^{3}H]-CGP 12177 (4 nM) that mainly labelled β_1/β_2-adrenoceptors. ICI 118 551 (a selective β_2-antagonist) and procaterol (a selective β_2-agonist) displaced (−)-[^{3}H]-CGP 12177 from its binding sites with very low affinity (K_i = 0.17 ± 0.02 μM and K_i = 11 ± 2 μM respectively).

6 From these observations, we conclude that: (1) two kinds of binding sites with low and high affinities for (−)-[^{3}H]-CGP 12177 can be detected in intact brown adipocytes, (2) there are 10 times more low than high affinity β-adrenoceptors, as determined by saturation or competition curve analysis, (3) the high affinity binding sites mainly correspond to β_1-adrenoceptors, whereas the low affinity sites represent β_3-adrenoceptors, and (4) β_2-adrenoceptors are undetectable.

7 It is suggested that the low affinity β_3-adrenoceptors represent the physiological receptors for noradrenaline secreted from sympathetic nerve endings when the concentration of the neurohormone in the synaptic cleft is very high and/or when the high affinity β_1-adrenoceptors are desensitized by prolonged sympathetic stimulation such as chronic cold exposure.

Keywords: β_1-, β_2-, β_3-adrenoceptors; brown adipose tissue; (−)-[^{3}H]-CGP 12177; CL 316 243; propranolol; bupranolol; catecholamines; CGP 20712A; adipocyte

Introduction

During the last decade, numerous pharmacological studies revealed the presence of atypical or β_3-adrenoceptors in a variety of tissues, and particularly in brown adipose tissue (BAT) (Arch *et al.*, 1984; Arch, 1989; Kaumann, 1989; Lafontan & Berlan, 1993). At present, there is little doubt that β_1-, β_2-, and β_3-adrenoceptors coexist in BAT. As a matter of fact, adrenoceptor mRNAs for β_1-, β_2-, and β_3-adrenoceptors have been identified in BAT (Revelli *et al.*, 1991; Granneman, 1992; Granneman & Lahners, 1992). Studies performed with rat isolated brown adipocytes have demonstrated that β_3-adrenoceptor agonists are as effective as noradrenaline for maximally stimulating thermogenesis (Bukowiecki *et al.*, 1980; 1981; Atgié *et al.*, 1991). However, the physiological function of the three β-adrenoceptor subtypes in mediating the metabolic effects of noradrenaline *in vivo*, still remains to be defined.

The principal goal of the present studies was to characterize the properties of β_1-, β_2-, and β_3-adrenoceptors in intact brown adipocytes. Although the presence of β_3-adrenoceptors has been detected in crude membrane preparations obtained from BAT homogenates (Muzzin *et al.*, 1992), the properties of β_3-adrenoceptors have not yet been determined in intact adipocytes or purified plasma membranes. It is known that typical brown adipocytes represent about 40% of the total cell population in BAT, the other cellular types being

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endothelial cells forming the numerous capillaries (50%), pericytes, interstitial stem cells, preadipocytes, protoadipocytes, mast cells etc. (remaining 10%) (Bukowiecki *et al.*, 1986; Géloën *et al.*, 1988; 1990; Goglia *et al.*, 1992). To avoid contamination by other cellular types, it was decided to assess the properties of β-adrenoceptors in intact brown adipocytes using the hydrophilic β-adrenoceptor radioligand, (−)-[^{3}H]-CGP 12177 (Staehelin *et al.*, 1983; Lacasa *et al.*, 1985; 1986; Levin & Sullivan, 1986; Mohell & Dicker, 1989).

Methods

Animals

Female Sprague-Dawley rats weighing 250–300 g were kept at 27°C for at least 2 weeks with a photoperiod of 12L:12D and fed Purina Chow *ad libitum*. Brown adipocytes were isolated, essentially as previously described (Bukowiecki *et al.*, 1981), from pooled interscapular BAT from 3 rats killed at 09 h 00 min. Briefly, cleaned pieces of tissue (about 1 g) were incubated for 15 min in a 2.5 ml Krebs-Ringer-bicarbonate buffer (KRB) (pH 7.4, gassed for 30 min with a 95% O_2:5% CO_2) containing bovine serum albumin (1%), glucose (2.7 mM), HEPES (20 mM) and collagenase (10 mg), with a shaking frequency of 160 cycles per min. At the end of the digestion period, the cells were filtered through a nylon filter (500 μm), diluted in 10 ml of buffer and centrifuged (80 *g*) at room temperature for 5 min. The floating cells were filtered again through a filter (200 μm) and centrifuged following the same procedure. The isolated adipocytes were finally washed twice with 3 ml of KRB and counted in a haemacytometer after trypan blue staining.

Saturation binding experiments

(−)-[^{3}H]-CGP 12177 was chosen for our binding assays because we found, in preliminary experiments, that it had a very low octanolic/aqueous partition coefficient (0.29 ± 0.01) compared to the classical β-adrenoceptor radioligand, [^{125}I]-iodocyanopindolol (24.63 ± 1.30), confirming previous observations (Lafontan *et al.*, 1987). In addition, binding at 25°C was rapid, saturable, proportional to cell concentration, and was not displaced by 0.1 mM phenylephrine (an α-agonist) or 5-hydroxytryptamine (5-HT), confirming previous observations (Levin & Sullivan, 1986; Staehelin *et al.*, 1983; Lacasa *et al.*, 1985; Mauriège *et al.*, 1988). The isolated fat cells were preincubated at 37°C for 15 min in a KRB buffer containing (l^{-1}): glucose 1 g, bovine serum albumin 5.5 g, chloroquine 10 mg (to inhibit ligand uptake and internalization), ascorbic acid 0.1, pargyline 10 mg, catechol 34 mg (to minimize the degradation of catecholamines) and GTP 60 mg (to convert all the β-adrenoceptors into a low affinity state), pH 7.4 (Lafontan *et al.*, 1987; Levitzki, 1986). The cells were then washed with the binding buffer and about $1-1.5 \times 10^5$ cells were incubated 30 min with different concentrations of (−)-[^{3}H]-CGP 12177 ranging from 0.1 to 130 nM in a final volume of 160 μl, essentially as described by Marette *et al.* (1993). However, in this study, we used the potent β-adrenoceptor antagonist, bupranolol (100 μM) (rather than propranolol) to assess nonspecific binding (see below). Incubation was stopped by adding 2 ml of ice-cold NaCl (0.9%) solution and followed by rapid vacuum filtration of the suspension through Whatmann GF/C filters placed on a Millipore manifold. The filters were then washed twice with 10 ml portions of an ice-cold NaCl solution. Radioactivity retained on filters was counted in 4 ml of scintillation liquid (Ecolite, ICN) with a beta counter at 35% efficiency.

Competition binding experiments

Cells ($1-1.5 \times 10^5$) were incubated at 25°C for 30 min with (−)-[^{3}H]-CGP 12177 (4 or 25 nM) in the presence of various agonists and antagonists. The radioligand was used either at a concentration of 4 nM to label preferentially the high affinity binding sites or at a concentration of 25 nM to recruit both the high and low affinity sites. At these concentrations, nonspecific binding assessed with 100 μM bupranolol represented about 13% and 32% of total binding, respectively. The values of nonspecific binding determined by Ligand programme analysis, when nonspecific binding was allowed to float, were similar to those determined with 100 μM bupranolol.

Data analysis

The EBDA programme was used to determine the Hill coefficient (n_H) and to generate a first approximation of the dissociation constant (K_D) (saturation analysis), the inhibition constant (K_i) (competition analysis) and the maximum number of binding sites (B_{max}). Then, the data were analysed by the non-linear curve fitting Ligand programme (Munson & Rodbard, 1980) to determine the final K_D, K_i and B_{max} values. The statistical method based on the 'extra sum of squares' principle (Munson & Rodbard, 1980) given by the Ligand programme was used to determine whether the curves were best fitted by a one- or two-site model. In saturation experiments, the nonspecific binding determined with bupranolol (100 μM) was fixed and all other constants were allowed to float. In competition studies, the K_D of the radioligand for high and low affinity sites were fixed as well as nonspecific binding determined with 100 μM bupranolol. Other constants were allowed to float. The dissociation constant of the different displacing drugs (K_i) were directly given by the Ligand programme. In all tables and figures, values represent means ± s.e. of 3–5 individual experiments performed on separate occasions. The horizontal dotted line in the figures represents the mean value of the nonspecific binding determined with 100 μM bupranolol and the shaded areas correspond to the s.e. ($n = 5$).

Drugs and chemicals

(−)-Noradrenaline bitartrate, (−)-adrenaline bitartrate, 5-HT, phenylephrine, bovine serum albumin (fraction IV) and collagenase (type II) were obtained from Sigma Chemicals Co. (St-Louis, MO, U.S.A.). GTP was purchased from Boehringer Mannheim (Laval, Québec, Canada) and (−)-[^{3}H]-CGP 12177 ((−)-4-(3-*t*-butylamino-2-hydroxypropoxy)-[5,7-^{3}H] benzimidazol-2-1)) (specific activity = 43 Ci mmol^{-1}) was obtained from Amersham (Oakville, Ontario, Canada). (−)-Propranolol and (−)-isoprenaline were purchased from RBI Biochemicals Inc. (Natick, MA, U.S.A.); CL 316 243 (disodium (**R**,**R**)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate) and (−)-bupranolol were kindly provided by Dr T.H. Claus (American Cyanamid Company, Lederle Laboratories, Pearl River, NY, U.S.A.) (Bloom *et al.*, 1992) and by Dr M. Lafontan (Université Paul Sabatier, Toulouse, France), respectively. The following compounds were provided as generous gifts: ICI 118 551 (erythro-(±)-1-(7-methyllindane-4-yloxy)-3-isopropylaminobutane-2-olhydrochloride) from ICI Pharmaceuticals (Mississauga, Canada) and CGP 20712A ((±)-(2-(3-carbomoyl-4-trifluormethyl-2-imidazolyl)-phenoxy)-2-propanolmethanesulphonate) from Ciba-Geigy (Mississauga, Canada). Procaterol (OPC-2009) (5-(1-hydroxy-2-isopropylaminobutyl)-8-hydrocarbostyril hydrochloride hemihydrate) (Yabuuchi *et al.*, 1977) was kindly provided by Otsuka Pharmaceuticals (Tokushima, Japan).

Results

Saturation binding studies

Saturation binding experiments with increasing concentrations of (−)-[^{3}H]-CGP 12177 ranging from 0.1 to 130 nM

revealed that the saturation curve was clearly biphasic (Figure 1). The first saturation appeared around 3 nM (see inset of Figure 1). Scatchard plots of the data in Figure 1 were curvilinear indicating that (−)-[^{3}H]-CGP 12177 bound to at least two classes of receptors (Figure 2). EBDA/Ligand analysis clearly indicated that the two-site model was more appropriate than the one-site model ($P<0.01$). Moreover, the Hill coefficient ($n_H = 0.57 \pm 0.11$) confirmed the presence of two different binding sites (Table 1). The low affinity adrenoceptors ($K_D = 80$ nM) were approximately 10 times more numerous than the high affinity adrenoceptors ($K_D = 0.24$ nM).

Competition binding studies with adrenoceptor agonists

Displacement curves were performed with concentrations of (−)-[^{3}H]-CGP 12177 fixed at 25 nM in order to label the high and low affinity binding sites. The capacity of isoprenaline, noradrenaline and adrenaline for displacing (−)-[^{3}H]-CGP 12177 from its binding sites was compared with that of a new selective β_3-agonist, CL 316 243 (Figure 3). All displacement curves of (−)-[^{3}H]-CGP 12177 were best fitted to a two-site model ($P<0.01$) with Hill coefficients smaller than 1 (Tables 2 and 3, Figures 3 and 4). Low concentrations of CL 316 243 (0.1–20 nM) displaced (−)-[^{3}H]-CGP 12177 with high efficiency from a predominant population of binding sites, whereas low concentrations of isoprenaline, noradrenaline or adrenaline displaced the radioligand from a minor population of sites. The results indicated that the major population of β_3-adrenoceptors had a much higher affinity for CL 316 243 than for catecholamines (a difference of 3–4 orders of magnitude), whereas the contrary was observed for the minor population of adrenoceptors. Phenylephrine, an α-adrenoceptor agonist and 5-HT did not displace (−)-[^{3}H]-CGP 12177 (25 nM) even when used at concentrations as high as 0.1 mM (data not shown).

Competition binding studies with an adrenoceptor antagonist

In order to characterize better the high affinity binding sites, competition experiments were performed with the selective β_1-antagonist, CGP 20712A. The displacement curve was biphasic with high (65 ± 19 pM) and low (3.8 ± 0.9 μM) K_is,

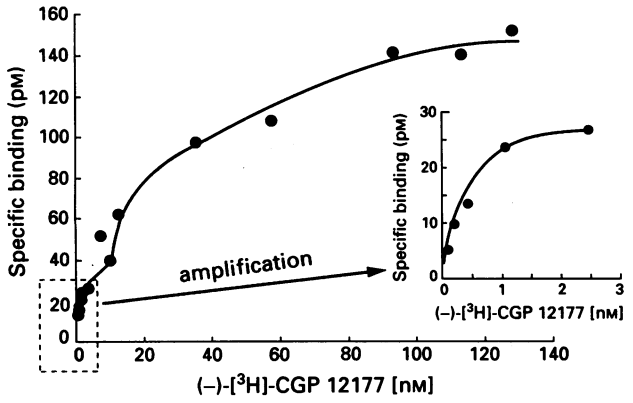

Figure 1 Saturation curve of (−)-[^{3}H]-CGP 12177 specific binding to intact brown adipocytes. Binding assays were performed as described in Methods. The inset amplifies the first saturation of high affinity sites from 0.10 to 2.5 nM of (−)-[^{3}H]-CGP 12177. The graph is from one representative experiment. The Hill coefficient (n_H), the dissociation constants (K_D) of the low and high affinity binding sites and the corresponding number of binding sites (B_{max}) calculated from 5 individual experiments are given in Table 1.

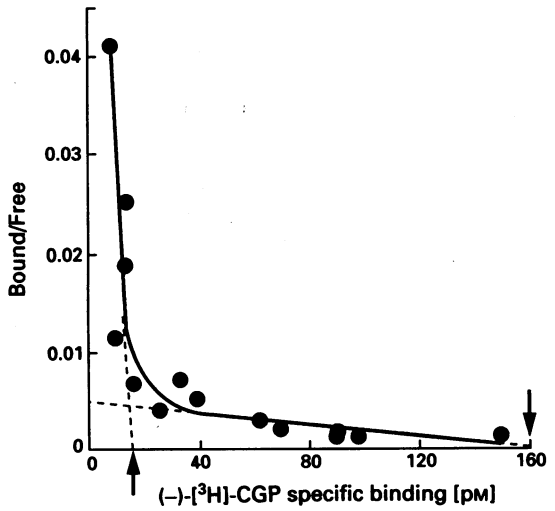

Figure 2 Scatchard analysis of the saturation curve presented in Figure 1. The biphasic plot ($P<0.01$) confirms the presence of at least two distinct populations of high (0.24 ± 0.04 nM) and low (80 ± 7 nM) affinity binding sites ($n = 5$).

Table 1 Pharmacological characteristics of (−)-[^{3}H]-CGP 12177 binding sites in intact rat brown adipocytes

	Dissociation constant K_D (nM)	*Number of sites* B_{max} (× 10^3 sites/cell)
High affinity sites	0.24 ± 0.04	12 ± 1
Low affinity sites	80 ± 7	121 ± 30

Saturation experiments were performed with intact brown adipocytes in a range of concentrations of (−)-[^{3}H]-CGP 12177 from 0.1 to 130 nM. The data were analysed by Scatchard transformation of the saturation curves by the EBDA/Ligand programme to determine the dissociation constant (K_D) and maximal number of binding sites (B_{max}). Values represent the mean ± s.e. of 5 individual experiments. The Hill coefficient (n_H) was 0.57 ± 0.11 ($P<0.01$).

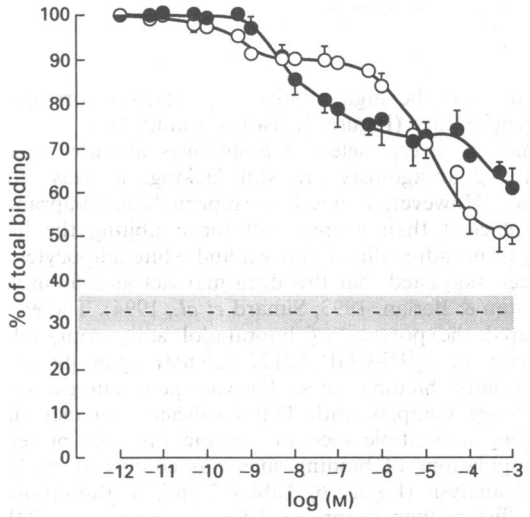

Figure 3 Displacement curves of (−)-[^{3}H]-CGP 12177 (25 nM) by isoprenaline (○) or by the selective β_3-agonist CL 316 243 (●). EBDA/Ligand analysis showed that biphasicity of both curves was statistically significant ($P<0.01$). The Hill coefficient (n_H) of the displacement curves, the inhibition constants (K_i) and the corresponding number of binding sites (B_{max}) are given in Tables 2 and 3. The values represent the mean ± s.e. of 3–5 individual experiments. Symbols without s.e. bars indicate that the s.e. is smaller than the diameter of the circle.

Table 2 Binding characteristics of different β-adrenoceptor drugs for β_1-adrenoceptors in rat intact brown adipocytes

Drugs	Hill coefficients n_H	Binding characteristics: Inhibition constant K_i (nM)	Binding characteristics: Inhibition constant pK_i	Number of sites B_{max} ($\times 10^3$ sites per cell)
Agonists				
Isoprenaline	0.41 ± 0.10 ($P<0.01$)	0.044 ± 0.008	10.4	8.7 ± 2.1
Noradrenaline	0.33 ± 0.10 ($P<0.01$)	0.47 ± 0.16	9.3	12 ± 3
Adrenaline	0.41 ± 0.13 ($P<0.01$)	2.0 ± 0.8	8.6	9.5 ± 1.3
CL 316 243	0.73 ± 0.06 ($P<0.01$)	3600 ± 1300	5.4	10 ± 1
Antagonists				
CGP 20712A	0.37 ± 0.04 ($P<0.01$)	0.065 ± 0.019	10.2	13 ± 3
Bupranolol	0.42 ± 0.11 ($P<0.01$)	0.029 ± 0.011	10.5	13 ± 1
Propranolol	0.46 ± 0.01 ($P<0.01$)	0.19 ± 0.07	9.7	10 ± 1

Competition studies were performed with intact brown adipocytes using a (−)-[^{3}H]-CGP 12177 concentration of 25 nM. Inhibition constants (K_i) and Hill coefficients (n_H) were evaluated by the EBDA/Ligand programme as described under Methods. Values represent the mean ± s.e. of 3–5 individual experiments.

Table 3 Binding characteristics of different β-adrenoceptor drugs for β_3-adrenoceptors in intact rat brown adipocytes

Drugs	Inhibition constant K_i (μM)	Inhibition constant pK_i	Number of sites B_{max} ($\times 10^3$ sites per cell)
Agonists			
CL 316 243	0.015 ± 0.004	7.8	71 ± 12
Isoprenaline	29 ± 10	4.5	131 ± 5
Noradrenaline	56 ± 3	4.2	157 ± 14
Adrenaline	91 ± 10	4.0	225 ± 14
Antagonists			
Bupranolol	0.27 ± 0.04	6.5	51 ± 11
Propranolol	1.6 ± 0.2	5.7	49 ± 5
CGP 20712A	3.8 ± 0.9	5.4	119 ± 21

The experimental conditions and the Hill coefficients were the same as in Table 2.

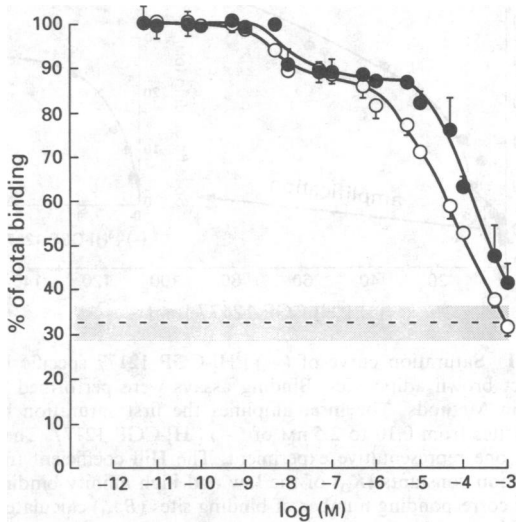

Figure 4 Displacement curves of (−)-[^{3}H]-CGP 12177 (25 nM) by noradrenaline (○) or by adrenaline (●). EBDA/Ligand analysis showed that biphasicity of both curves was statistically significant ($P<0.01$). The Hill coefficient (n_H) of the displacement curves, the inhibition constants (K_i) and the corresponding number of binding sites (B_{max}) are given in Tables 2 and 3. For other details see Figure 3.

attesting that the high affinity binding sites correspond to β_1-adrenoceptors (Figure 5, Tables 2 and 3).

Although several selective β_3-agonists are now available, selective β_3-antagonists are still lacking, at least for the moment. However, it has been reported that bupranolol is more efficient than propranolol for inhibiting the lipolytic effects of noradrenaline in brown and white adipocytes and it has been suggested that this drug may act as a β_3-antagonist (Lafontan & Berlan, 1993; Simard *et al.*, 1994). Therefore, we compared the potency of bupranolol and propranolol for displacing (−)-[^{3}H]-CGP 12177 (25 nM) from its low and high affinity binding sites. Displacement curves, in both cases, were complex with Hill coefficients smaller than 1, indicating a multiple receptor interaction. The presence of two populations of binding sites was confirmed by EBDA-Ligand analysis (Figure 6, Tables 2 and 3). Bupranolol was more efficient than propranolol for displacing (−)-[^{3}H]-CGP 12177 from its high affinity binding sites ($K_i = 0.029 \pm 0.011$ nM and 0.19 ± 0.07 nM, $P<0.01$, unpaired t test). The affinities of bupranolol and propranolol for the second population of adrenoceptors were much lower but bupranolol was still more efficient than propranolol for displacing the radioligand ($K_i = 0.27 \pm 0.04$ μM and 1.6 ± 0.2 μM, $P<0.01$, unpaired t test). Thus, CGP 20712A, bupranolol and propranolol all displaced with high affinity (−)-[^{3}H]-CGP 12177 from the small population of binding sites. However, propranolol and bupranolol generated displacement curves without an apparent transition between the high and low affinity binding sites (Figure 6), whereas the displacement curve obtained with CGP 20712A was clearly biphasic. The higher affinity of CGP 20712A for β_1-adrenoceptors could explain that difference.

Competition studies with selective β_2-adrenoceptor agents

To assess whether β_2-adrenoceptors could be detected in the population of high affinity binding sites, the capacity of two selective β_2-agents, ICI 118 551 (an antagonist) and procaterol (an agonist), for displacing (−)-[^{3}H]-CGP 12177 was evaluated (Figure 7). A reduced concentration of (−)-[^{3}H]-CGP 12177 (4 nM) was used to label preferentially the high

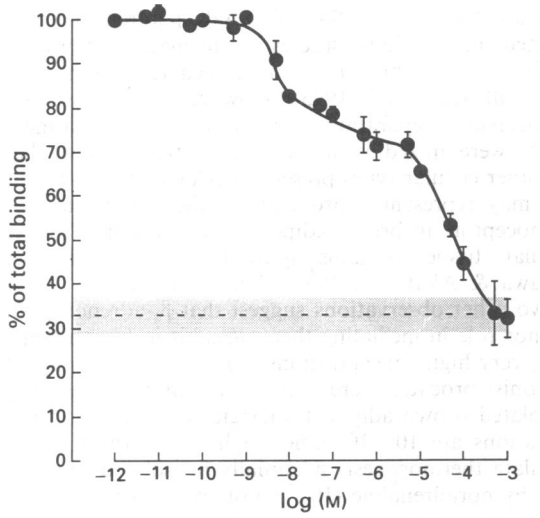

Figure 5 Displacement curve of (−)-[^{3}H]-CGP 12177 (25 nM) by the selective β_1-antagonist, CGP 20712A. EBDA/Ligand analysis showed that the curve was best fitted to a two-site model ($P<0.01$). The Hill coefficient (n_H) of the displacement curves, the inhibition constants (K_i) and the corresponding number of binding sites (B_{max}) are given in Tables 2 and 3. For other details see Figure 3.

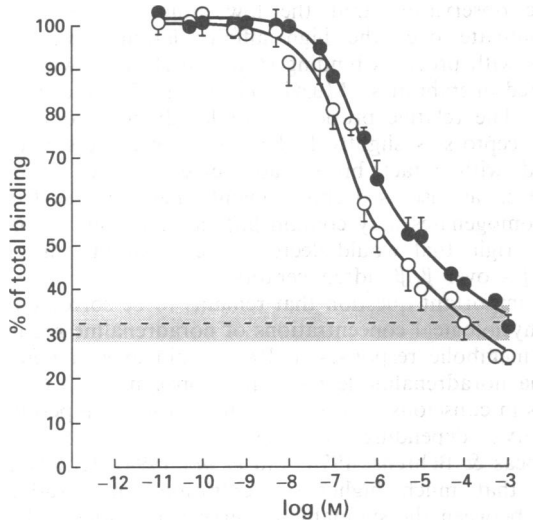

Figure 6 Displacement curve of (−)-[^{3}H]-CGP 12177 (25 nM) by the nonselective β-antagonists propranolol (●) and bupranolol (○). EBDA/Ligand analysis showed that biphasicity of both curves was statistically significant ($P<0.01$). The Hill coefficient (n_H) of the displacement curves, the inhibition constants (K_i) and the corresponding number of binding sites (B_{max}) are given in Tables 2 and 3. For other details see Figure 3.

affinity binding sites (see inset of Figure 1). ICI 118 551 and procaterol displaced (−)-[^{3}H]-CGP 12177 only at very high concentrations with elevated K_i valules (0.17 ± 0.02 μM and 11 ± 2 μM, respectively). Radioligand binding analysis revealed that the displacement curves for both ICI 118 551 and procaterol were best fitted to a one-site model ($P<0.01$). The B_{max} values ($12\,000 \pm 2\,000$ and $22\,000 \pm 4\,000$ sites per cell for procaterol and ICI 118 551) were within the same range of values as the high affinity B_{max} obtained from saturation studies with (−)-[^{3}H]-CGP 12177 (Figures 1, 2 and Table 1). Thus, β_2-adrenoceptors were not detectable.

Discussion

The main objective of the present studies was to characterize the properties of β-adrenoceptors in intact brown adipocytes.

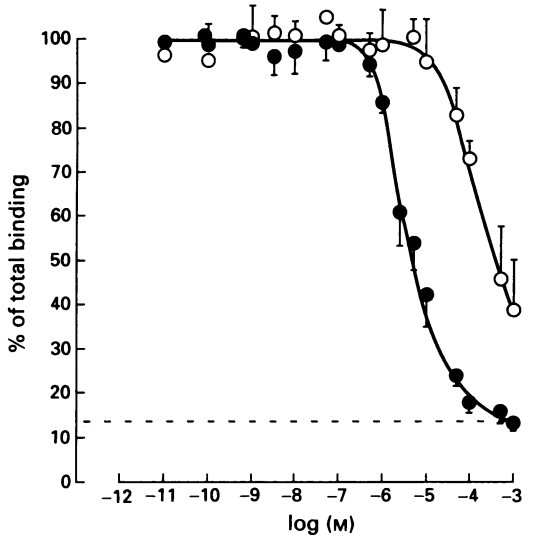

Figure 7 Displacement curve of (−)-[^{3}H]-CGP 12177 (4 nM) by the selective β_2-antagonist, ICI 118 551 (●) and the selective β_2-agonist procaterol (○). EBDA/Ligand analysis showed that the data best fitted to a one-site model ($P<0.01$). The Hill coefficient (n_H) of the displacement curves, the inhibition constants (K_i) and the corresponding number of binding sites (B_{max}) are given in Table 4.

Histological cellular frequency studies have demonstrated that typical brown adipocytes represent about 40% of the total cell population in BAT (Géloën *et al.*, 1988). Endothelial cells forming the numerous capillaries surrounding brown adipocytes occupy another 50%, the remaining 10% being distributed between interstitial stem cells, protoadipocytes, preadipocytes, mast cells etc. It is likely that the distribution of β-adrenoceptors differs among these cellular types and that it changes during BAT growth, cold acclimatization, etc. Vascular tissue contains both β_1- and β_2-adrenoceptors (Aikawa & Akatsuka, 1990; Zink *et al.*, 1993) and may represent a major contaminant in membrane preparations obtained from whole tissue homogenates.

Previous studies, performed with membrane preparations obtained from tissue homogenates, have used radioligands such as (−)-[^{3}H]-dihydroalprenolol or (−)-[^{125}I]-cyanopindolol to determine the properties of β-adrenoceptor-binding sites (Bukowiecki *et al.*, 1978; Dax & Partilla, 1982; Senault *et al.*, 1984; Raasmaja, 1990; Langin *et al.*, 1991; Galitzky *et al.*, 1993b). However, these two radioligands were inadequate for quantifying β-adrenoceptors in intact cells due to their high lipophilicity. Therefore, we chose the highly hydrophilic radioligand (−)-[^{3}H]-CGP 12177 (octanolic/aqueous partition coefficient = 0.29 ± 0.01) to analyses the properties of β-adrenoceptors in intact brown adipocytes. (−)-[^{3}H]-CGP 12177 has been used successfully for quantifying β_1- and β_2-adrenoceptors in intact rat and human adipocytes (Lacasa *et al.*, 1985; 1986; Lafontan *et al.*, 1987; Levin & Sullivan, 1986; Mauriège *et al.*, 1988; Mohell & Dicker, 1989; Marette *et al.*, 1993), but it is the first time that it has been used for characterizing the properties of β_3-adrenoceptors in isolated brown adipocytes. In addition, nonspecific binding was determined with the potent β-antagonist bupranolol (Kaumann, 1989). Bupranolol is 10 times more potent than propranolol for inhibiting the lipolytic effects of β_3-agonists in white (Lafontan & Berlan, 1993) and brown (Simard *et al.*, 1994) adipocytes.

Both saturation (Figures 1 and 2) and competition (Figures 3–7) studies revealed that the low affinity β_3-adrenoceptors predominate over the high affinity β_1-adrenoceptors in brown adipocytes and that β_2-adrenoceptors are undetectable. Adrenoceptor agonists displaced (−)-[^{3}H]-CGP 12177 from its high affinity sites with a potency order typical of β_1-adrenoceptors: isoprenaline≫ noradrenaline>

adrenaline≫CL 316 243≫phenylephrine (Table 2 and Figures 3–6) (Mohell & Nedergaard, 1989). Likewise, the same agents displaced the radioligand from the low affinity sites with a potency order corresponding to β_3-adrenoceptors: CL 316 243≫isoprenaline>noradrenaline>adrenaline≫phenylephrine (Table 3 and Figures 3 and 4) (Fève *et al.*, 1991). Furthermore, the K_D values of the high affinity β_1-adrenoceptors (0.24 nM) and low affinity β_3-adrenoceptors (80 nM) for (−)-[^{3}H]-CGP 12177 (Table 1) were in the same range of values as the corresponding K_D observed in CHO cells expressing the rat β_3-adrenoceptor (Muzzin *et al.*, 1992), in 3T3-F442A adipocytes (Fève *et al.*, 1991), in BAT homogenates (Muzzin *et al.*, 1992), and in garden dormouse white adipocytes (Carpéné *et al.*, 1994).

It may be argued that the K_i values for catecholamines (Table 2) are higher than those previously reported for β_1-adrenoceptors (Levin & Sullivan, 1986; Mohell & Nedergaard, 1989; Raasmaja, 1990). However, most of the past studies have been performed on membranes (and not on cells) and generally used concentrations of displacing drugs ranging from 10^{-9} to 10^{-4} M (and not 10^{-12} to 10^{-4}) in the presence of concentrations of radioligand that bound β_1-, β_2- and probably β_3-adrenoceptors. Under these conditions, the K_i values represent a mean of the K_is for the three β-adrenoceptor subtypes. In fact, we also found, in preliminary experiments, that the K_i value for noradrenaline is in the 10^{-7} M range using *membranes* and [^{125}I]-cyanopindolol (200 pM), but we believe that this is an overestimation for the above reasons.

Noradrenaline displaced (−)-[^{3}H]-CGP 12177 from β_3-adrenoceptors with low affinity (K_i = 56 μM), suggesting that it may stimulate thermogenesis in BAT via both the high (β_1) and low affinity (β_3) receptors. It is clear that the β_3-adrenoceptors will be recruited only at very high noradrenaline concentrations because noradrenaline affinity for β_1-adrenoceptors is several fold greater than for β_3-adrenoceptors (Tables 2 and 3). This marked difference in binding affinities agrees with the difference in noradrenaline capacity for stimulating adenylate cyclase activity, lipolysis or respiration via the high and low affinity receptors (Granneman, 1990; Atgié *et al.*, 1991; 1994; Simard *et al.*, 1994).

It has been reported in functional studies that bupranolol is 10 times more potent than propranolol for inhibiting the lipolytic effects of β_3-agonists (Lafontan & Berlan, 1993; Simard *et al.*, 1994). The displacement curves described in Figure 6 also showed that bupranolol is a better β-antagonist than propranolol. The K_i values of burpanolol and propranolol are comparable to the K_i or pA_2 values reported in literature (Muzzin *et al.*, 1992; Galitzky *et al.*, 1993a).

In order to evaluate further the proportion of β_1- over β_2-adrenoceptors, competition studies were carried out with a β_2-antagonist (ICI 118 551) and a β_2-agonist (procaterol). These experiments revealed that the high affinity β-adrenoceptor binding sites for (−)-[^{3}H]-CGP 12177 mainly represented β_1-adrenoceptors because β_2-selective drugs were not effective in displacing the radioligand from its binding sites (Figure 7 and Table 4). The presence of β_2-adrenoceptors (30–50% of total β-adrenoceptors) was detected in previous studies performed with membranes obtained from whole tissue homogenates (Rothwell *et al.*, 1985; Sillence *et al.*, 1993). However, in the whole tissue homogenate preparations, brown adipocyte plasma membranes were mixed with membranes from endothelial cells and other cellular types present in BAT (Géloën *et al.*, 1988). This may represent a problem for the quantification of β_2-adrenoceptors in brown adipocytes because it is known that vascular tissue contains both β_1- and β_2-adrenoceptors (Aikawa & Akatsuka, 1990; Zink *et al.*, 1993).

Two other observations suggest that β_2-adrenoceptors play a minor role in mediating thermogenesis in brown adipocytes. First, very high concentrations (10^{-5}–10^{-4} M) of the selective β_2-agonist procaterol are required to stimulate thermogenesis in isolated brown adipocytes (Atgié *et al.*, 1994). These concentrations are 10^2–10^3 times higher than those required to stimulate thermogenesis maximally in isolated brown adipocytes by noradrenaline (10^{-7} M) or by the selective β_3-agonist BRL 37344 (10^{-8} M) (Bukowiecki *et al.*, 1980; 1981; Atgié *et al.*, 1991; 1994). Second, the β_2-adrenoceptor antagonist, ICI 118 551, is only a weak inhibitor of the lipolytic or respiratory effects of noradrenaline. Thus, the present binding data as well as our recent respiratory studies suggest that β_2-adrenoceptors are much less important than β_1- or β_2-adrenoceptors for mediating the respiratory effects of noradrenaline in rat brown adipocytes.

The observation that the low affinity β_3-adrenoceptors predominate over the high affinity β_1-adrenoceptors also agrees with previous binding studies performed with unfractionated membranes (Muzzin *et al.*, 1992; Sillence *et al.*, 1993). The relative proportion of β_3-adrenoceptors over β_1-adrenoceptors is slightly higher in the present studies conducted with intact brown adipocytes (Tables 2 and 3). However, as discussed above, membranes prepared from tissue homogenates may contain β_1/β_2-adrenoceptors from vascular origin that would decrease the apparent value of the ratio β_3- over β_1/β_2-adrenoceptors.

An important question that remains to be solved concerns the physiological concentrations of noradrenaline required to elicit metabolic responses in BAT. Cold exposure increases plasma noradrenaline levels from approximately 1 nM (basal values in conscious undisturbed rats at room temperature) to 3–10 nM, depending on the temperature of exposure (Depocas & Behrens, 1978; Liu *et al.*, 1994). However, it is likely that much higher concentrations of noradrenaline occur between the sympathetic nerve varicosities and brown adipocyte plasma membrane, particularly after intensive stress. Unlike white adipose tissue, BAT is densely innervated with sympathetic nerves that run, not only along the capillaries, but also between the individual adipocytes (Cottle *et al.*, 1985). Indirect evidence suggests that concentrations of noradrenaline as high as 100 nM may occur in the synaptic cleft (Depocas *et al.*, 1978). One hundred nanomolar is approximately the noradrenaline concentration that is required to stimulate thermogenesis maximally in isolated brown adipocytes (Bukowiecki *et al.*, 1980; 1981). On the

Table 4 Binding characteristics of different β_2-adrenoceptor agents for high affinity (−)-[^{3}H]-CGP 12177 binding sites in rat intact brown adipocytes

	Binding characteristics			
Drugs	*Hill coefficients* n_H	*Inhibition constant* K_i (μM)	*Inhibition constant* pK_i	*Number of sites* B_{max} (× 10^3 sites per cell)
ICI 118 551	0.99 ± 0.11 ($P<0.01$)	0.17 ± 0.02	6.7	22 ± 2
Procaterol	0.85 ± 0.28 ($P<0.01$)	11 ± 2	4.9	12 ± 2

Competition studies were performed with intact brown adipocytes using a (−)-[^{3}H]-CGP 12177 concentration of 4 nM. Other experimental conditions were the same as in Table 2.

other hand, it is known that β_1-adrenoceptors may be desensitized (internalized or down-regulated) by chronic cold exposure (Bukowiecki *et al.*, 1978) or prolonged exposure to β-agonists (Granneman, 1992). In contrast, β_3-adrenoceptors are particularly resistant to catecholamine-induced desensitization (Granneman, 1992; Carpéné *et al.*, 1993). It should also be pointed out that the affinity of β_3-adrenoceptors for noradrenaline is of the same order of magnitude as the affinity of receptors for neurotransmitters such as acetylcholine (10–100 μM) (Changeux, 1987). All these observations suggest that β_3-adrenoceptors represent the physiological receptors for noradrenaline secreted from sympathetic nerve endings, i.e. when the concentration of the neurohormone in the synaptic cleft is high and/or when the high affinity β_1-adrenoceptors are desensitized by prolonged sympathetic stimulation. In this context, the main role of the high affinity β_1-adrenoceptors would be to mediate the effects of circulating noradrenaline (at low nanomolar levels). Further studies are required to test these hypotheses directly.

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References

AIKAWA, J. & AKATSUKA, N. (1990). Vascular smooth muscle relaxation by endothelium-dependent β_1-adrenergic action. *Comp. Biochem. Physiol.*, **97C**, 311–315.

ARCH, J.R.S. (1989). The brown adipocyte beta-receptors. *Proc. Nutr. Soc.*, **48**, 215–223.

ARCH, J.R.S., AINSWORTH, A.T., CAWTHORNE, M.A., PIERCY, V., SENNITT, M.V., THODY, V.E., WILSON, C. & WILSON, S. (1984). Atypical beta-adrenoceptors on brown adipocytes as target for anti-obesity drugs. *Nature*, **309**, 163–165.

ATGIÉ, C., MARETTE, A. & BUKOWIECKI, L.J. (1991). Norepinephrine and BRL 37344 stimulate thermogenesis in rat brown adipose tissue via different receptors. *Int. J. Obesity*, **15S**, 52.

ATGIÉ, C., SIMARD, P.-M., D'ALLAIRE, F. & BUKOWIECKI, L.J. (1994). Relative contribution of β_1- and β_3-adrenoceptors to the regulation of lipolysis and respiration in rat brown adipocytes. *FASEB J.*, **8**, A960.

BLOOM, J.D., DUTIA, M.D., JOHNSON, B.D., WISSNER, A., BURNS, M.G., LARGIS, E.E., DOLAN, J.A. & CLAUS, T.H. (1992). Disodium(R,R)-5-[2-[[2-(3-chlorophenyl)-2-hydroxy-ethyl)-amino]-propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL 316 243): a potent beta-adrenergic agonist virtually specific for the beta$_3$-adrenoceptors; a promising antidiabetic and antiobesity agent. *J. Med. Chem.*, **35**, 3081–3084.

BUCKOWIECKI, L.J., FOLLÉA, N., LUPIEN, J. & PARADIS, A. (1981). Metabolic relationships between lipolysis and respiration in rat brown adipocytes. *J. Biol. Chem.*, **256**, 12840–12848.

BUKOWIECKI, L.J., FOLLÉA, N., PARADIS, A. & COLLET, A.J. (1980). Stereospecific stimulation of brown adipose tissue respiration by caetcholamines via beta-adrenergic receptors. *Am. J. Physiol.*, **238** (Endocrinol. Metab. Vol. 1), E552–E563.

BUKOWIECKI, L.J. FOLLÉA, N., VALLIERES, J. & LEBLANC, J. (1978). β-adrenergic receptors in brown-adipose tissue. Characterization and alterations during acclimation of rats to cold. *Eur. J. Biochem.*, **92**, 189–196.

BUKOWIECKI, L.J., GÉLOËN, A. & COLLET, A.J. (1986). Proliferation and differentiation of brown adipocytes from interstitial cells during cold acclimation. *Am. J. Physiol.*, **250** (Cell Physiol. Vol. 19), C880–C887.

CARPÉNÉ, C., AMBID, L. & LAFONTAN, M. (1994). Predominance of β3-adrenergic component in catecholamine activation of lipolysis in garden dormouse adipocytes. *Am. J. Physiol.*, **266** (Regulatory Integrative Comp. Physiol. Vol. 35), R896–R904.

CARPÉNÉ, C., GALITZKY, J., COLLON, P., ESCLAPEZ, F., DAUZATS, M. & LAFONTAN, M. (1993). Desensitization of beta-1 and beta-2, but not beta-3, adrenoceptor-mediated lipolytic responses of adipocytes after long-term norepinephrine infusion. *J. Pharmacol. Exp. Ther.*, **265**, 237–247.

CHANGEUX, J.-P. (1987). Acetylcholine receptor. In *Encyclopedia of Neuroscience*, ed. Adelman, G. pp. 2–3. Boston: Birkhäusser.

COTTLE, M.K.W., COTTLE, W.H., PÉRUSSE, F. & BUKOWIECKI, L.J. (1985). An imposed glyoxylic acid technique for the histochemical localization of catecholamines in brownadipose tissue. *Histochem. J.*, **17**, 1279–1288.

DAX, E.M. & PARTILLA, J.S. (1982). Adrenergic ligand liposolubility in membranes: direct assessment in a beta-adrenergic binding system. *Mol. Pharmacol.*, **22**, 5–7.

DEPOCAS, F. & BEHRENS, W.A. (1978). Levels of noradrenaline in plasma during thermogenesis induced by cold-exposure or by noradrenaline infusion in warm- and in cold-acclimated rats. *Exp. Suppl.*, **32**, 135–146.

DEPOCAS, F., BEHRENS, W.A. & FOSTER, D.O. (1978). Noradrenaline-induced calorigenesis in warm- and in cold-acclimated rats: the interrelation of dose of noradrenaline, its concentration in arterial plasma, and calorigenic response. *Can. J. Physiol. Pharmacol.*, **56**, 168–174.

FÈVE, 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 receptors. *J. Biol. Chem.*, **266**, 20329–20336.

GALITZKY, J., REVERTE, M., CARPÉNÉ, C., LAFONTAN, M. & BERLAN, M. (1993a). β3-Adrenoceptors in dog adipose tissue: studies on their involvement in the lipomobilizing effect of catecholamines. *J. Pharmacol. Exp. Ther.*, **266**, 358–366.

GALITZKY, J., REVERTE, M., PORTILLO, M., CARPÉNÉ, C., LAFONTAN, M. & BERLAN, M. (1993b). Coexistence of β_1-, β_2-, β_3-adrenoceptors in dog fat cells and their differential activation by catecholamines. *Am. J. Physiol.*, **264** (Endocrinol. Metab. Vol. 27), E403–E412.

GÉLOËN, A., COLLET, A.J., GUAY, G. & BUKOWIECKI, L.J. (1988). β-adrenergic stimulation of brown adipocyte proliferation. *Am. J. Physiol.*, **254** (Cell Physiol. Vol. 23), C175–C182.

GÉLOËN, A., COLLET, A.J., GUAY, G. & BUKOWIECKI, L.J. (1990). In vivo differentiation of brown adipocyte in adult mice: an electron microscopic Study. *Am. J. Anatomy*, **188**, 366–372.

GOGLIA, F., GÉLOËN, A., LANNI, A., MINAIRE, Y. & BUKOWIECKI, L.J. (1992). Morphometric-stereologic analysis of brown adipocyte differentiation in adult mice. *Am. J. Physiol.*, **262** (Cell Physiol. Vol. 31), C1018–C1023.

GRANNEMAN, J.G. (1990). Norepinephrine and BRL 37344 stimulate adenylate cyclase by different receptors in rat brown adipose tissue. *J. Pharmacol. Exp. Ther.*, **254**, 508–513.

GRANNEMAN, J.G. (1992). Effects of agonist exposure on the coupling of beta 1 and beta 3 adrenergic receptors to adenylyl cyclase in isolated adipocytes. *J. Pharmacol. Exp. Ther.*, **261**, 638–642.

GRANNEMAN, J.G. & LAHNERS, K.N. (1992). Differential adrenergic regulation of beta 1- and beta 3-adrenoreceptor messenger ribonucleic acids in adipose tissues. *Endocrinology*, **130**, 109–114.

KAUMANN, A.J. (1989). Is there a third heart β-adrenoceptor? *Trends Pharmacol. Sci.*, **10**, 316–320.

LACASA, D., AGLI, B. & GIUDICELLI, Y. (1985). Direct assessment of beta-adrenergic receptors in intact rat adipocytes by binding of [^{3}H]-CGP-12177. Evidence for agonist high affinity complex and for β_1- and β_2-receptors subtypes. *Eur. J. Biochem.*, **146**, 339–346.

LACASA, D., MAURIÈGE, P., LAFONTAN, M., BERLAN, M. & GIUDICELLI, Y. (1986). A reliable assay for β-adrenoceptors in intact isolated human fat cells with a hydrophilic radioligand, [^{3}H]-CGP 12177. *J. Lipid Res.*, **27**, 368–376.

LAFONTAN, M. & BERLAN, M. (1993). Fat cell adrenergic receptors and the control of white and brown fat cell function. *J. Lipid Res.* (Review), **34**, 1057–1091.

LAFONTAN, M., MAURIÈGE, P., DE PERGOLA, G., GALITZKI, J. & BERLAN, M. (1987). Identification and quantification of beta and alpha 2 adrenoceptors on membranes and intact adipocytes. In *Proceeding of the 5th International Congress on Obesity, Recent Advances in Obesity Research: V.* ed. Berry, E.M., Blondheim, S.H., Eliahou, H.E. & Shafrir, E. pp. 212–221. London: John Libbey & Company.

LANGIN, D., PORTILLA, 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.

LEVITZKI, A. (1986). β-adrenergic receptors and their mode of coupling to adenylate cyclase. *Physiol. Rev.*, **66**, 819–854.

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

LIU, X., PÉRUSSE, F. & BUKOWIECKI, L.J. (1994). Chronic norepinephrine infusion stimulates glucose uptake in white and brown adipose tissues. *Am. J. Physiol.*, **266** (Regulatory Integrative Comp. Physiol. Vol. 35), R914–R920.

MARETTE, A., MAURIÈGE, P., DESPRÉS, J.-P., TULP, O.L. & BUKOWIECKI, L.J. (1993). Norepinephrine- and insulin-resistant glucose transport in brown adipocytes from diabetic SHR/N-cp rats. *Am. J. Physiol.*, **265** (Regulatory Integrative Comp. Physiol. Vol. 34), R577–R583.

MAURIÈGE, P., DE PERGOLA, G., BERLAN, M. & LAFONTAN, M. (1988). Human fat cell beta-adrenergic receptors: beta-agonist dependent lipolytic responses and characterization of beta-adrenergic binding sites on human fat cell membranes with highly selective beta-1-antagonists. *J. Lipid Res.*, **29**, 587–601.

MOHELL, N. & DICKER, A. (1989). The β-adrenergic radioligand [^{3}H] CGP 12177, generally classified as an antagonist, is a thermogenic agonist in brown adipose tissue. *Biochem. J.*, **261**, 401–405.

MOHELL, A. & NEDERGAARD, J. (1989). Comparison of the pharmacological profiles of adrenergic drugs (including BRL-agonists) at [^{3}H]prazosin and [^{3}H] CGP12177 binding sites in brown adipose tissue. *Comp. Biochem. Physiol.*, **94C**, 229–233.

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

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

RAASMAJA, A. (1990). Alpha 1- and beta-adrenergic receptors in brown adipose tissue and the adrenergic regulation of thyroxine 5'-deiodinase. *Acta Physiol. Scand.*, **139** (suppl. 590), 1–64.

REVELLI, J.P., PESCINI, R., MUZZIN, P., SEYDOUX, J., FITZGERALD, M.G., FRASER, C.M. & GIACOBINO, J.P. (1991). Changes in beta 1- and beta 2-adrenergic receptor mRNA levels in brown adipose tissue and heart of hypothyroid rats. *Biochem. J.*, **277**, 625–628.

ROTHWELL, N.J., STOCK, M.J. & SUDERA, D.K. (1985). β-adrenoceptors in rat brown adipose tissues: proportions of β_1- and β_2-subtypes. *Am. J. Physiol.*, **248** (Endocrinol. Metab. Vol. 11), E397–E402.

SENAULT, C., LE COMTE, V. & PORTET, R. (1984). Characteristics of β-adrenergic receptors in isolated cells and in crude membranes of brown adipose tissue. *Biochimie*, **66**, 573–578.

SILLENCE, M.N., MOORE, N.J., PEGG, G.G. & LINDSAY, D.B. (1993). Ligand binding properties of putative β_3-adrenoceptors compared in brown adipose tissue and skeletal muscle membranes. *Br. J. Pharmacol.*, **109**, 1157–1163.

SIMARD, P.M., ATGIÉ, C., MAURIÈGE, P., D'ALLAIRE, F. & BUKOWIECKI, L.J. (1994). Comparison of the lipolytic effects of norepinephrine and BRL 37344 in rat brown and white adipocytes. *Obesity Res.*, **2**, 424–431.

STAEHELIN, M., SIMONS, P., JAEGG, K. & WIGGER, N. (1983). A hydrophilic β-adrenergic receptor radioligand reveals high affinity binding of agonist to intact cells. *J. Biol. Chem.*, **258**, 3496–3502.

YABUUCHI, Y., YAMASHITA, S. & TEI, S.S. (1977). Pharmacological studies of OPC 2009, a newly synthesized β-adrenoceptor stimulant in the bronchomotor and cardiovascular system of the anesthetized dog. *J. Pharmacol. Exp. Ther.*, **202**, 326–336.

ZINK, S., RÖSEN, S., SACKMANN, B. & LEMOINE, H. (1993). Regulation of endothelial permeability by β-adrenoceptor agonists: contribution of β1- and β2-adrenoceptors. *Biochim. Biophys. Acta*, **1178**, 286–298.

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