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Characterization of a New, Highly Specific, β_3-Adrenergic Receptor Radioligand, [^{3}H]SB 206606

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SUMMARY

The *RR*-enantiomer of the β_3-adrenergic receptor agonist BRL 37344 was tritiated to yield a high specific activity compound, [^{3}H]SB 206606. This new, potentially specific, β_3-adrenergic receptor ligand was characterized by binding studies using membranes from both Chinese hamster ovary K1 cells transfected with the rat β_3-adrenergic receptor and rat interscapular brown adipose tissue, where β_1-, β_2-, and β_3-adrenergic receptor subtypes are known to coexist. [^{3}H]SB 206606 was found to bind to a single population of binding sites in both preparations. The K_d values for [^{3}H]SB 206606 binding to membranes from Chinese hamster ovary K1 cells and brown adipose tissue were quite comparable (58 and 38 nM, respectively). At 37°, the time courses of association and dissociation of [^{3}H]SB 206606 with membranes of brown adipose tissue were quite short. At 4°, the $T_{1/2}$ were found to be 13 and 40 min, respectively. The K_i values for various β-adrenergic agonists and antagonists in brown adipose tissue membranes were similar to those obtained in Chinese hamster ovary K1 cell membranes with both [^{3}H]SB 206606 and [^{125}I]iodocyanopindolol. The order of binding affinity was BRL 37344 ≫ (−)-isoproterenol = (−)-norepinephrine > (−)-epinephrine = (+)-isoproterenol. The similarity of the K_d values and of the K_i values for various β-adrenergic agonists and antagonists in both systems tested indicates that, in a complex membrane system, [^{3}H]SB 206606 binds selectively to the β_3-adrenergic receptor. The affinity of [^{3}H]SB 206606 is 76 times higher for the β_3-adrenergic receptor than for the β_1/β_2-adrenergic receptors, thus allowing, under controlled conditions, measurement of interactions only with the β_3-adrenergic receptor in complex membrane systems.

An atypical β-AR called the β_3-AR has been recently cloned starting from human and mouse genomic DNA (1, 2) and rat cDNA (3, 4) libraries. This new β-AR subtype was found to be expressed in rodent brown and white adipose tissues (3, 4), in 3T3-F442A adipocytes (5), and in colon (6). Pharmacologically, as described previously in rat brown adipose tissue (7), the β_3-AR expressed in transfected cells is characterized by a high affinity for thermogenic β_3-AR agonists such as BRL 37344 and a low affinity for the classical β-AR antagonists such as (−)-propranolol and (−)-alprenolol (1–5, 8).

Until now, two β-AR radioligands, [^{125}I]ICYP and [^{3}H]CGP 12177, have been used to characterize ligand-receptor interactions in cells transfected with the β_3-AR (1, 2, 4, 5). High concentrations of ligand had to be used because the affinities of [^{125}I]ICYP and [^{3}H]CGP 12177 for the β_3-AR are low, compared with those for the β_1/β_2-AR (1, 2, 4, 5). In the case of fat cell membranes, where the β_3-AR coexists with the β_1- and β_2-AR, measurement of the binding of the lipophilic radioligand [^{125}I]ICYP to the β_3-AR was found to be difficult because of the high level of nonspecific binding (9). It was only when the hydrophilic radioligand [^{3}H]CGP 12177, which is a β_1/β_2-AR antagonist and a β_3-AR agonist (10), was used that measurement of the binding to the β_3-AR in IBAT plasma membranes could be performed (11). However, this measurement necessitates a complex experimental protocol, i.e., complete ligand saturation and competition displacement analyses, because [^{3}H]CGP 12177 binds not only to the β_3-AR but also to the β_1/β_2-AR subtypes. It would thus be of interest to obtain a ligand that would bind selectively to the β_3-AR.

For this purpose, the *RR*-enantiomer of the β_3-AR agonist BRL 37344, which is known to bind with a higher affinity to the β_3-AR than to the β_1/β_2-AR, was tritiated to yield a high specific activity ligand, [^{3}H]SB 206606. The characteristics of [^{3}H]SB 206606 binding to both CHO-K1 cells transfected with

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ABBREVIATIONS: AR, adrenergic receptor(s); ICYP, iodocyanopindolol; IBAT, interscapular brown adipose tissue; CHO, Chinese hamster ovary; HPLC, high performance liquid chromatography.

the rat β_3-AR and rat IBAT membranes are described in the present study.

Experimental Procedures

Materials

All organic and inorganic chemicals were of analytical or molecular biology grade. (−)-[^{125}I]ICYP and (−)-[^{3}H]CGP 12177 were purchased from Amersham. CHO-K1 cells were from the American Type Culture Collection, and tissue culture reagents were from GIBCO/BRL. Sprague-Dawley male rats (9 weeks of age) were kept at room temperature (about 20°) with 12 hr of illumination each day and were fed *ad libitum* with Provimi Lacta chow (Cossonay, Switzerland).

Chemical Syntheses

(*RR*)-Methyl-2,6-diiodo-4-[(2-(2-(3-chlorophenyl)-2-hydroxyethyl)amino)propyl]phenoxy acetate. Methyl-2,6-diiodo-4-[(2-(2-(3-chlorophenyl)-2-hydroxyethyl)amino)propyl]phenoxy acetate was prepared, as a mixture of diastereomers, from 3,5-diiodobenzaldehyde by a procedure analogous to that described for BRL 35135 (12, 13) [^{1}H NMR ($CDCl_3$): δ 1.25 (3 H, d), 2.3–3.8 (7 H, complex), 3.85 (3 H, s), 4.6 (2 H, s), 4.8 (1 H, t), 7.0–7.6 (4 H, complex), and 7.75 (2 H, s]. The mixture of diastereomers was separated by preparative HPLC using a Dynamax 60A silica 83–121-C (250 × 21 mm; Rainin Instruments Co.) column and 12% ethanol (containing 0.1% triethylamine)/88% hexane as mobile phase (flow rate, 12 ml/min; detection, UV absorbance at 254 nm). The *RS,SR*-diastereomer had a retention time of 19.6 min (97% purity) and the *RR,SS*-diastereomer had a retention time of 21.8 min (95% purity). The *RR,SS*-diastereomer was separated into individual enantiomers by preparative chiral HPLC using a Chiralcel OJ (250 × 10 mm; Daicel Industries) column and 10% ethanol/90% hexane as mobile phase (flow rate, 2 ml/min; detection, UV absorbance at 254 nm). The *RR*-enantiomer had a retention time of 64 min (100% purity) and the *SS*-enantiomer had a retention time of 77 min (99% purity). BRL 37344, which is a racemic mixture of *RR*- and *SS*-enantiomers, as well as pure *RR*- (BRL 44092) and *SS*- (BRL 45182) enantiomers, were used in this study.

(*RR*)-4-[(2-2-(3-chlorophenyl)-2-hydroxyethyl)amino)-propyl][^{3}H]phenoxyacetic acid (SB 206606). (*RR*)-Methyl-2,6-diiodo-4-[(2-(2-(3-chlorophenyl)-2-hydroxyethyl)amino)propyl]phenoxy acetate (5 mg) was dissolved in anhydrous dimethylformamide (1 ml), 5% palladium on calcium carbonate catalyst (2.5 mg) was added, and the mixture was agitated under an atmosphere of tritium gas (5 Ci) for 5 hr (performed by Amersham International plc). After filtration, the labile tritium was removed *in vacuo* using conventional techniques. Purification was effected by preparative HPLC using a Spherisorb 5ODS2 (4.6 × 250 mm) column with 70% methanol/30% 0.05 M aqueous ammonium formate as eluant. After lyophilization, the residue was redissolved in a mixture of tetrahydrofuran and water. Aqueous sodium hydroxide solution was added portionwise (1.5 mg/ml; total of ~6 equivalents), and the mixture was warmed to 40°, until the hydrolysis was complete, and then diluted with aqueous ethanol (1:1, v/v; 5 ml). Hydrochloric acid (0.01 M, 1.2 ml) was added, followed by aqueous ammonium formate (1 M, 1 ml), and the solution was lyophilized. The residue was purified by preparative HPLC using a Spherisorb 5ODS2 (4.6 × 250 mm) column and 45% methanol/55% 0.05 M aqueous ammonium formate as eluant. The lyophilized product (total activity, 10.8 mCi) was redissolved in degassed ethanol/water (3:7, v/v; 6.35 ml). Direct chemical ionization mass spectrometry using ammonia provided the following data: *m/z* (percentage relative intensity) 364 (9.84), 366 (21.7), 368 (30), and 370 (8.2); this yielded a calculated specific activity of 36.5 Ci/mmol. Proton-decoupled 400-MHz ^{3}H NMR spectroscopy (ethanol/water/D_2O) showed a single resonance at 6.95 ppm. The radiochemical purity was determined to be 94.8% by HPLC. The chemical structure of [^{3}H]SB 206606 is shown in Fig. 1.

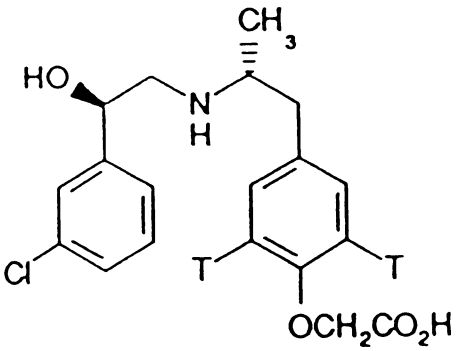

Fig. 1. Chemical structure of [^{3}H]SB 206606.

Lipolysis Studies

Glycerol release from rat IBAT (14) was measured over 30 min in the presence of 4% bovine serum albumin, as described previously (7). EC_{50} values are expressed relative to the maximal effect of the agonist.

Binding Studies

The IBAT plasma membranes were prepared from pools of IBAT from six rats as described previously (15), and lung crude membranes were prepared from the lungs of one rat by the method of Rugg *et al.* (16). Protein concentrations were determined by the method of Lowry *et al.* (17). The cloning of the rat cDNA encoding the β_3-AR, its stable expression in CHO-K1 cells, and the preparation of CHO-K1 cell membrane fractions have been described (4).

In radioligand binding studies, 50 μg of membrane proteins were incubated for 30 min at 37° in 50 mM Tris·HCl, pH 7.4, 10 mM $MgCl_2$ (total volume, 0.5 ml), containing 300 μM GTP and the indicated amounts of [^{3}H]SB 206606, [^{3}H]CGP 12177, or [^{125}I]ICYP. Ascorbic acid (1 mM) and pyrocatechol-3,5-disulfonic acid (Tiron) (1 μM) were systematically added to the incubation medium. These compounds are useful in protecting the catechol group of β-AR agonists when the latter are used in displacement studies (18). The binding of the radioligand to the membranes was stopped by dipping of the tube into an ice bath and was determined by filtration at 4° using a Brandel M-24 R apparatus. Specific binding was defined as the difference between the total binding obtained in the absence of competing ligand and the nonspecific binding obtained in the presence of (−)-isoproterenol (5 mM). (−)-Propranolol (100 μM) and (±)-CGP 12177 (50 μM) were also tested as unlabeled competitors. The results were identical to those obtained using (−)-isoproterenol (data not shown). Each assay was performed in duplicate. The quantitative parameters (K_d and B_{max}) were determined by Scatchard analysis using the LIGAND program (19). The K_d value was utilized in computer analysis of competition displacement curves.

Results

The nonlabeled equivalent of [^{3}H]SB 206606, i.e., the pure *RR*-enantiomer of the β_3-AR agonist BRL 37344, was first tested for its agonist properties. It was found to stimulate lipolysis in isolated rat IBAT adipocytes with an EC_{50} value of 3.3 ± 0.8 nM (nine experiments). BRL 37344, which is a racemic mixture of both the *RR*- and *SS*-enantiomers, had an EC_{50} value of 6.7 ± 1.2 nM (10 experiments). The ratio of the EC_{50} value of the *RR*-enantiomer to that of BRL 37344 was 2.0, which corresponds to the theoretical value. The nonlabeled *SS*-enantiomer had a very high EC_{50} of 10,022 ± 2,429 nM (nine experiments).[1] The *RR*-enantiomer of BRL 37344 is a full

[1] M. Oriowo, H. Chapman, D. M. Kirkham, M. V. Sennitt, R. R. Ruffolo, and M. A. Cawthorne, unpublished observations.

agonist, i.e., has an intrinsic activity similar to that of (−)-isoproterenol.

Fig. 2 shows the results of [^{3}H]SB 206606 binding studies performed using IBAT membranes. It can be seen that the specific [^{3}H]SB 206606 binding increased linearly with the amount of membrane proteins in a range of concentrations varying from 10 to 200 μg/assay. The time course of [^{3}H]SB 206606 interaction with the β_3-AR is illustrated in Fig. 3. At 37° (Fig. 3A), the association time course was quite short, with 77% of the equilibrium binding value being established by the earliest point tested (1 min). Binding at this temperature was also rapidly reversible. When the incubation medium was diluted after equilibrium binding had been established, 93% of the ligand dissociated by 1 min. The same study was performed at 4° (Fig. 3B). The time courses were slower under this condition. The half-times of association and dissociation were about 13 ± 2 min (three experiments) and 40 ± 10 min (three experiments), respectively.

Fig. 4 shows the results of the [^{3}H]SB 206606 binding studies performed using rat β_3-AR-transfected CHO-K1 cell (Fig. 4A) or IBAT (Fig. 4B) membranes. The specific binding of [^{3}H]SB 206606, measured in a range of concentrations varying from 2 nM to 150 nM, was found to be saturable. The nonspecific binding value, obtained in the presence of 5 mM (−)-isoproterenol, was found to increase linearly with increasing concentrations of the ligand. At 40 nM [^{3}H]SB 206606 in CHO-K1-β_3 cell and IBAT membranes, the value had a mean of 28% and 52%, respectively, of the total binding value. Scatchard analysis of saturation isotherms indicated the presence of a single population of binding sites in the CHO-K1-β_3 and IBAT membrane preparations, with Hill coefficients of 0.98 ± 0.01 and 0.93 ± 0.07, respectively. As shown in Table 1, the K_d values for [^{3}H]SB 206606 binding to CHO-K1-β_3 and IBAT membranes (58 ± 14 and 38 ± 16 nM, respectively) were quite comparable.

Fig. 5 shows the displacement of [^{3}H]SB 206606 binding to IBAT membranes by increasing concentrations of various β-AR agonists. The sequence of binding affinity was BRL 37344 ≫ (−)-isoproterenol = (−)-norepinephrine > (−)-epinephrine = (+)-isoproterenol. Table 2 shows the K_i values for inhibition of [^{3}H]SB 206606 binding to IBAT membranes, as calculated from the displacement curves shown in Fig. 5. It also shows K_i values for inhibition of [^{3}H]SB 206606 binding to CHO-K1-β_3 cell membranes. These values were quite similar to each other for each β-AR agonist tested. Table 2 also shows K_i values for inhibition of [^{125}I]ICYP binding to CHO-K1-β_3 cell membranes. Values presented for (−)-isoproterenol and (−)-norepinephrine were obtained in new experiments performed using a protocol identical to that used for [^{3}H]SB 206606. Values presented for BRL 37344 and (−)-epinephrine are those already reported by Muzzin *et al.* (4). The results show a great similarity in the K_i values obtained with either [^{3}H]SB 206606 or [^{125}I]ICYP.

The K_i values for inhibition of [^{3}H]SB 206606 binding by various β-AR antagonists are also shown in Table 2. The K_i values for (−)-propranolol were quite similar in CHO-K1-β_3 cell and IBAT membranes and, in CHO-K1-β_3 cell membranes, using either [^{3}H]SB 206606 or [^{125}I]ICYP as labeled ligand.

The possibility that [^{3}H]SB 206606, under the conditions used to measure its interaction with the β_3-AR in complex membrane systems, might also bind to other β-AR subtypes was tested. For this purpose a membrane fraction prepared from rat lung, which has been shown to contain only the β_1- and β_2-AR, with the β_2-AR being predominant (16), was used. At a concentration of 150 nM [^{3}H]SB 206606, no specific binding could be measured in this preparation (data not shown). The displacement of [^{3}H]CGP 12177 (2 nM) binding to lung membranes by increasing concentrations of BRL 37344 was also studied. The K_i value for the inhibition of [^{3}H]CGP 12177 binding to lung β_1/β_2-AR calculated from the displacement curve was 6.4 μM (two experiments). This K_i value is 76 times greater than that for the inhibition by BRL 37344 of [^{3}H]SB 206606 binding to the IBAT β_3-AR (Table 2). Together, these results show that the affinity of [^{3}H]SB 206606 for the β_3-AR is sufficiently greater than that for the β_1/β_2-AR to allow, under controlled conditions, measurement of interaction with only the β_3-AR in complex membrane systems.

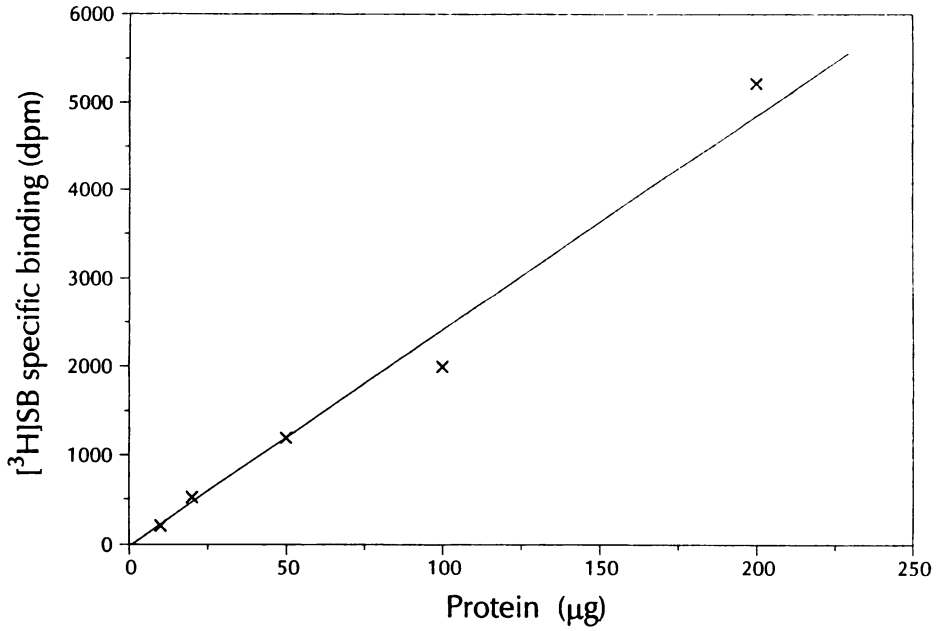

Fig. 2. Specific binding of [^{3}H]SB 206606 (*SB*) (40 nM) to rat IBAT plasma membranes as a function of increasing amounts of proteins. The results are the mean of two experiments and are expressed as specific binding.

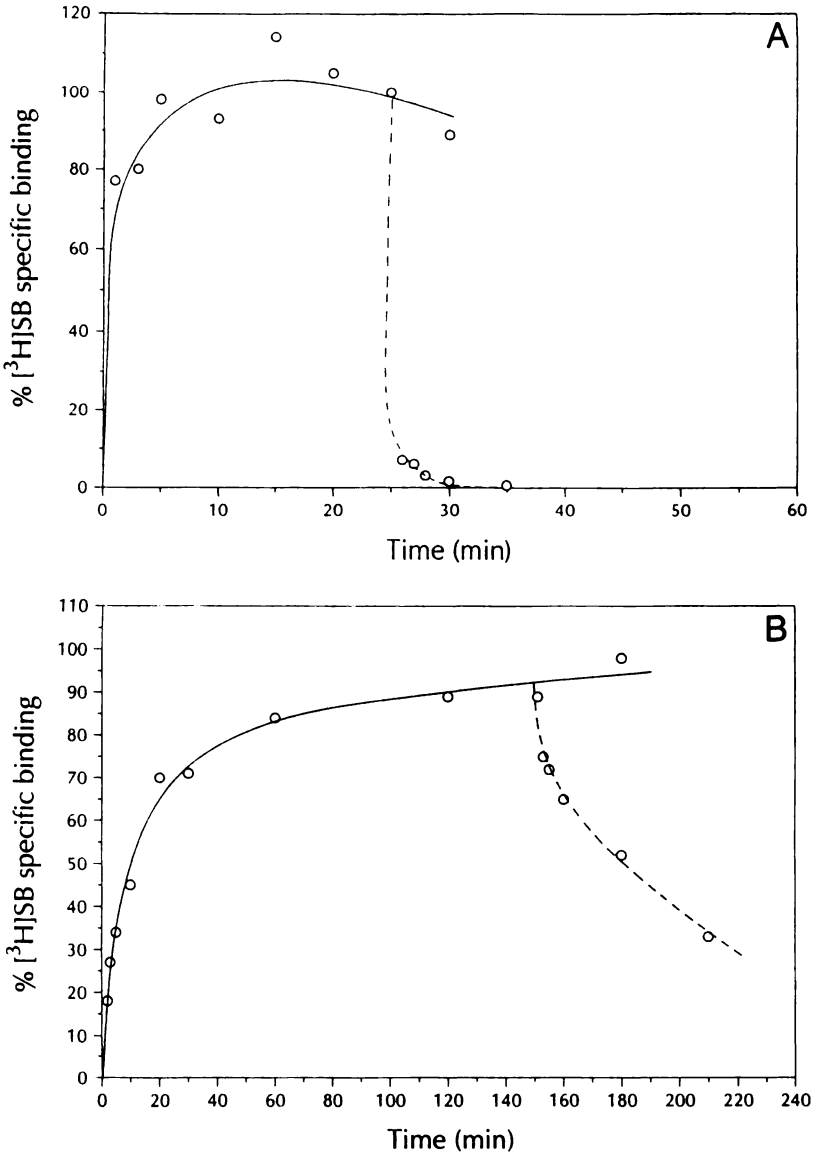

Fig. 3. Time courses of ligand association (solid lines) and dissociation (dashed lines) at 37° (A) and 4° (B) for specific binding of [^{3}H]SB 206606 (*SB*) (40 nM) to rat IBAT plasma membranes. Reversibility of binding was measured, after equilibrium had been established, by diluting the membranes 20-fold with 50 mM Tris·HCl, pH 7.4, 10 mM $MgCl_2$, at the indicated temperature. The results illustrated are from one representative experiment and are expressed as a percentage of specific binding at equilibrium.

It was of interest to confirm that [^{3}H]SB 206606 is a better ligand to measure binding to the β_3-AR in complex membrane systems, where this receptor coexists with the β_1- and β_2-AR, than is the classical β-AR ligand [^{125}I]ICYP. For this purpose, the displacement by BRL 37344 of [^{125}I]ICYP binding to IBAT plasma membranes was measured. [^{125}I]ICYP was used at a concentration of 0.8 nM, i.e., near its previously determined K_d value for the β_3-AR, using cells transfected with the β_3-AR (1, 2, 4, 5). BRL 37344 was used at a concentration of 1 μM, i.e., well above its K_i value for the β_3-AR that was determined in this study (see Table 2). The displacement by BRL 37344 was found to amount to only 8% of [^{125}I]ICYP total binding. This indicates that, at the high concentrations of [^{125}I]ICYP required to bind not only to the β_1- and β_2-AR but also to the β_3-AR subtypes in IBAT membranes, the part of the total binding due to specific interaction with the β_3-AR is too small to allow for its accurate measurement. This finding confirms the results of previous studies (9) showing that [^{125}I]ICYP cannot be used to measure binding to the β_3-AR in complex membrane systems. [^{125}I]ICYP, however, is a good ligand to perform binding studies in CHO-K_1-β_3 cells.

Discussion

Lipolytic studies performed with the *RR*-enantiomer BRL 44092 indicated that [^{3}H]SB 206606 behaves as a pure β_3-AR agonist. The binding properties of this compound were then studied. The fact that only one population of binding sites was detected in IBAT membranes, with a K_d similar to that obtained in CHO-K_1-β_3 cell membranes, suggests that, in the complex IBAT membrane system, [^{3}H]SB 206606 binds selectively to the β_3-AR in the range of concentrations used (2–150 nM).

The binding of [^{3}H]SB 206606 was found to be rapid and rapidly reversible at 37° in IBAT membranes. High rates of association and dissociation have also been reported for [^{3}H] dihydroalprenolol (20) and [^{125}I]ICYP (21) in frog erythrocyte and guinea pig lung membranes, respectively, at 37°.

β-AR belong to a class of receptors that mediate their action by coupling to G proteins. The formation of agonist-β-AR-G protein complex is characterized by an increase in receptor affinity for agonist. GTP destabilizes this complex, converting the high affinity state receptors to low affinity state receptors (for review, see Ref. 22). It is not known whether the existing

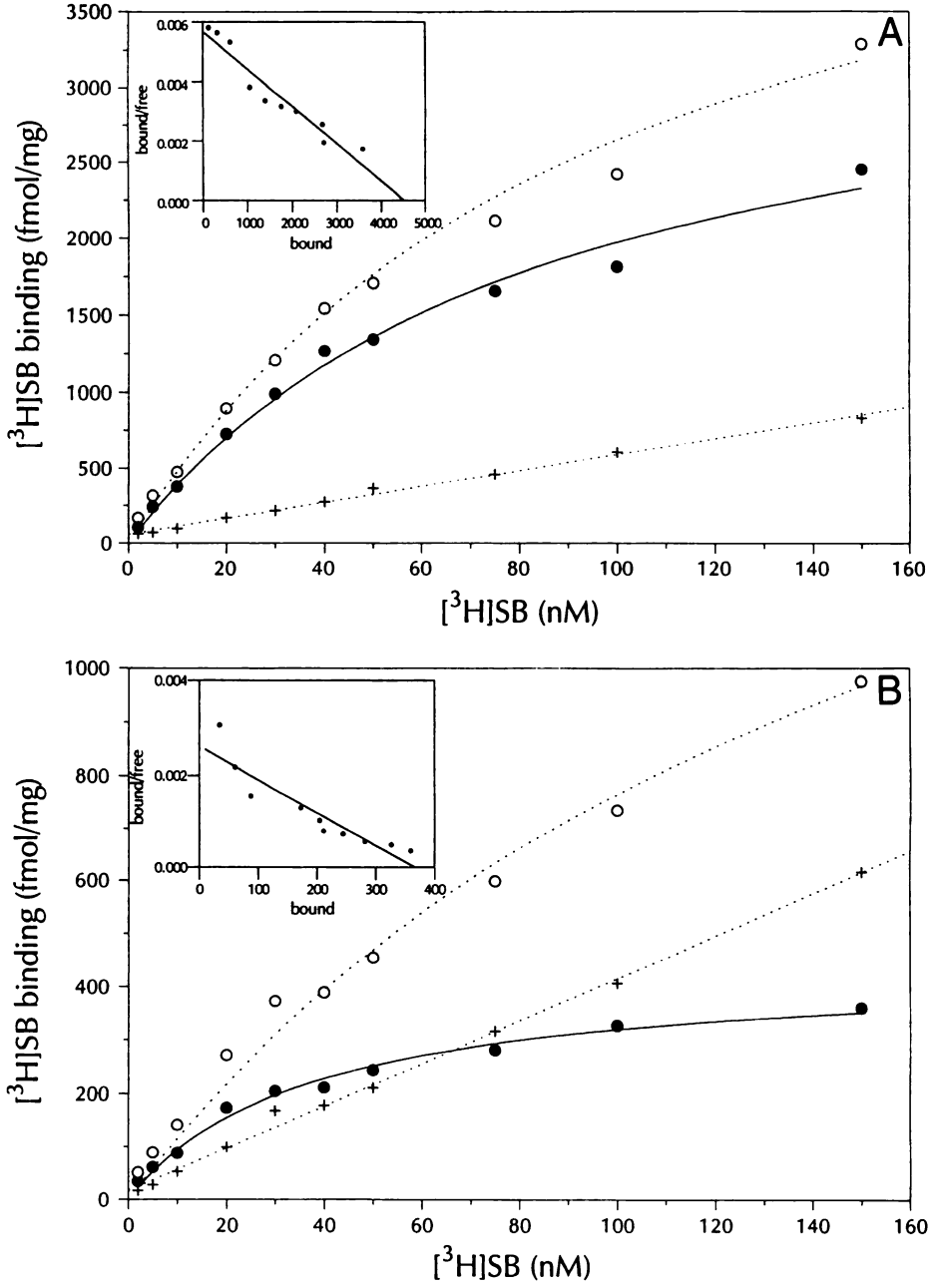

Fig. 4. Total binding (○), nonspecific binding (+), and specific binding (●) of [^{3}H]SB 206606 (*SB*) to CHO-K1-β_3 cell membranes (A) and to rat IBAT plasma membranes (B) as a function of increasing concentrations of the ligand. The results illustrated are from one representative experiment and are expressed as fmol of ligand bound/mg of cell membrane proteins. *Insets*, Scatchard analyses of the data from the representative experiment.

TABLE 1

Binding of [^{3}H]SB 206606 to CHO-K1-β_3 cell or rat IBAT membranes

The B_{max} values are expressed as fmol of ligand bound/mg of cell membrane proteins. The values are the mean ± standard error of the number of experiments in parentheses. Concentrations of [^{3}H]SB 206606 used varied from 2 to 150 nM. Nonspecific binding values were obtained in the presence of 5 mM (−)-isoproterenol.

	K_d	B_{max}
	nM	fmol/mg
CHO-K1-β_3	58 ± 14 (3)	3424 ± 1431 (3)
IBAT	38 ± 16 (4)	417 ± 130 (4)

ligand [^{125}I]ICYP is an agonist or an antagonist of the β_3-AR, because the functional action of ICYP has not been studied with this receptor. Despite this lack of knowledge, most workers have included GTP in their assay and this was also done in the present study. It is presently not known whether the β_3-AR mediates its action via a classical G protein. [^{3}H]SB 206606 will be a good tool to test whether agonist binding to the β_3-AR is GTP sensitive. This study is presently being performed in our laboratory.

Displacement studies were then performed using [^{3}H]SB 206606 and various agonists or antagonists, in both CHO-K_1-β_3 cell and IBAT membranes. The great similarity of the K_i values obtained in the two systems confirms that [^{3}H]SB 206606 binds only to β_3-AR. The sequence of binding affinity was found to be BRL 37344 ≫ (−)-isoproterenol = (−)-norepinephrine > (−)-epinephrine = (+)-isoproterenol. The K_i for [^{125}I]ICYP displacement by (−)-norepinephrine obtained in this study is 1 order of magnitude smaller than that reported previously by Muzzin *et al.* (4) in the same cell system. Fève *et al.* (5), in cells transfected with the human β_3-AR and in 3T3-F442A cells, have observed that (−)-isoproterenol is more potent than (−)-norepinephrine in displacing [^{125}I]ICYP binding.

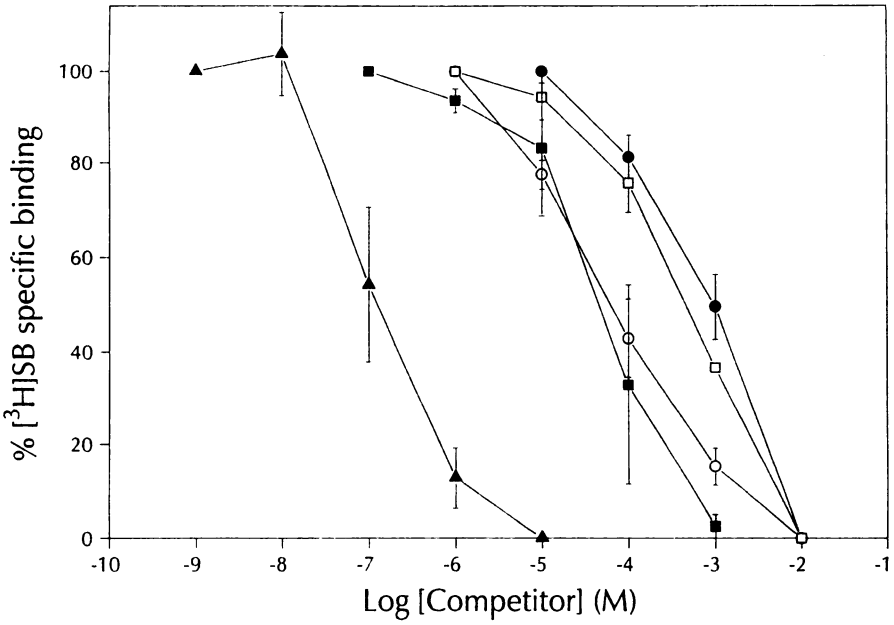

Fig. 5. Displacement of [^{3}H]SB 206606 (*SB*) (40 nM) binding to rat IBAT plasma membranes by increasing concentrations of BRL 37344 (▲), (−)-isoproterenol (○), (−)-norepinephrine (■), (−)-epinephrine (□), and (+)-isoproterenol (●). The results illustrated are the mean ± standard error of three or four experiments and are expressed as a percentage of specific binding in the absence of competitor.

TABLE 2

Inhibition of [^{3}H]SB 206606 or [^{125}I]ICYP binding (K_i) to CHO-K1-β_3 cell or rat IBAT membranes by various β-AR agonists or antagonists

The values are the mean ± standard error of the number of experiments in parentheses. The concentrations of [^{3}H]SB 206606 and [^{125}I]ICYP used were 40 nM and 0.8 nM, respectively.

	K_i		
	[^{3}H]SB 206606		[^{125}I]ICYP, CHO-K1-β_3
	CHO-K1-β_3	IBAT	
		μM	
BRL 37344	0.112 ± 0.011 (3)	0.084 ± 0.031 (3)	0.250 ± 0.040[a]
(−)-Isoproterenol	17.4 ± 5.6 (3)	11.5 ± 3.2 (3)	45.3 ± 9.0 (3)
(−)-Norepinephrine	20.7 ± 8.9 (3)	27.1 ± 14.2 (4)	46.3 ± 12.8 (3)
(−)-Epinephrine	101 ± 15 (3)	432 ± 218 (3)	160 ± 80[a]
(+)-Isoproterenol		515 ± 198 (3)	
(±)-Cyanopindolol		0.18 ± 0.04 (3)	
(±)-CGP 12177		0.30 ± 0.11 (3)	
(−)-Propranolol	3.87 ± 1.88 (3)	1.35 ± 0.27 (3)	1.50 ± 1.80[a]

[a] Data from Muzzin *et al.* (4).

Ligget (8), in cells transfected with the rat β_3-AR, reported that (−)-isoproterenol is equipotent with (−)-norepinephrine in displacing [^{125}I]ICYP binding. The present study, using [^{3}H]SB 206606, is the first to show the sequence of binding affinity of various β-AR agonists for the β_3-AR in a complex biological system. It shows that the pharmacology of the rat β_3-AR in brown adipose tissue is very comparable to that already reported in transfected cells (8). It is also noteworthy that the K_i value obtained for BRL 37344 in this study is comparable to that obtained in [^{3}H]CGP 12177 binding studies (11).

The K_i value of (±)-CGP 12177 obtained for IBAT membranes is very close to that obtained for cells transfected with rat β_3-AR in [^{125}I]ICYP binding studies (8) and, as expected, much higher than the K_d for the binding of [^{3}H]CGP 12177 to IBAT β_1- or β_2-AR (11). The K_i values of (−)-propranolol, which are quite similar in CHO-K$_1$-β_3 cell and IBAT membranes, are, as expected, much higher than those reported for (−)-propranolol interaction with the β_1- or β_2-AR (11). The only discrepancy observed with previous data for the β_3-AR in transfected cells is the K_i of (±)-cyanopindolol, which is much higher than the K_d of [^{125}I]ICYP binding reported previously (4). This discrepancy might be due to the difference in structure and conformation of the two compounds, due to the iodination of cyanopindolol (23).

Comparison in both CHO-K1-β_3 cell and IBAT membranes of the [^{3}H]SB 206606 K_d value (Table 1) with the BRL 37344 K_i value (Table 2) shows that the *RR*-enantiomer has an affinity for the β_3-AR that is 2.0 and 2.2 times higher, respectively, than that of the racemic mixture of *RR*- and *SS*-enantiomers, BRL 37344. The K_i value for inhibition of [^{3}H]SB 206606 binding to IBAT membranes by the *RR*-enantiomer was 50 nM (data not shown), i.e., very close to the [^{3}H]SB 206606 K_d value. Thus, purification of the *RR*-enantiomer from the racemic mixture does not increase the affinity more than the theoretical factor of 2.

The affinity of binding of BRL 37344 is 76 times higher for the β_3-AR than for the β_1/β_2-AR. The same difference should exist between the affinities of [^{3}H]SB 206606 for the β_3- and β_1/β_2-AR. It is noteworthy that K_i values of BRL 37344 for β_3- and β_1/β_2-AR, obtained previously (11) using [^{3}H]CGP 12177 and IBAT membranes, were similar to those observed in this study. It is thus most unlikely that, under the conditions used

to measure the interaction of [^{3}H]SB 206606 with the β_3-AR, significant binding to the β_1/β_2-AR might occur. This is confirmed by the fact that, in this study, no specific binding of [^{3}H]SB 206606 could be observed in lung membranes even at high concentrations (150 nM) of the ligand. It is known that BRL 37344 and therefore [^{3}H]SB 206606 at high concentrations bind also to the β_1/β_2-AR (11). What is new regarding [^{3}H]SB 206606, compared with all labeled β-AR ligands presently used, is the fact that it binds with higher affinity to the β_3-AR than to the β_1/β_2-AR. It is thus possible, using [^{3}H]SB 206606, to directly measure the β_3-AR in complex biological systems.

It is noteworthy that, when K_{act} values for the accumulation of cAMP in CHO-K1 cells transfected with human β_3- and β_2-AR were compared, the affinity of BRL 37344 for the β_3-AR was found to be only 3.4 times higher than that for the β_2-AR (24). The reason for this difference might be due to the fact that the affinity of BRL 37344 for the rat β_3-AR is much higher than its affinity for the human β_3-AR (8). This must be kept in mind when [^{3}H]SB 206606 is used to measure binding to the β_3-AR in human tissues.

In conclusion, a new labeled β_3-AR agonist, [^{3}H]SB 206606, is described that binds with higher affinity to the β_3-AR than to the β_1/β_2-AR. This ligand allows for direct measurement of the β_3-AR in complex biological systems.

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