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Stereoselective Action of (*R**,*R**)-(±)-Methyl-4-[2-[2-hydroxy-2-(3-chlorophenyl)ethylamino]propyl]-phenoxyacetic Acid (BRL37344) on β-Adrenoceptors and Metabolic Chiral Inversion

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ABSTRACT. Stereoisomers of BRL37344 ((*R**,*R**)-(±)-methyl-4-[2-[2-hydroxy-2-(3-chlorophenyl)ethylamino]propyl]-phenoxyacetic acid), a β_3-adrenoceptor agonist, were synthesized and separated with good resolution by derivatization with 1-anthroyl cyanide prior to chiral HPLC. Agonist effects on rat right atria, guinea pig trachea, and rat brown adipocytes were due principally to the (*RR*) isomer, while other isomers (*SS*, *RS*, and *SR*) were much less potent or inactive. Since the racemate (*RR* + *SS*) was half as potent as the (*RR*) isomer in all specimens tested, the (*SS*) isomer does not appear to have antagonistic effects. When [^{14}C](*RR*)BRL35135A ((*R**,*R**)-(±)-methyl-4-[2-[2-hydroxy-2-(3-chlorophenyl)ethylamino]propyl]-phenoxyacetate hydrobromide), the HBr salt of the methyl ester of BRL37344, was administered orally to male Wistar rats, both the (*RR*) and (*SR*) isomers of [^{14}C]BRL37344 were detected in plasma, while only the (*SS*) isomer of [^{14}C]BRL37344 was detected after [^{14}C](*SS*)BRL35135A administration. These findings indicate that there is clear stereoselectivity in the effects of BRL37344 on β-adrenoceptors, and that stereoselective chiral inversion from the *RR* isomer to the *SR* isomer occurs in rats. BIOCHEM PHARMACOL 52;10:1521–1527, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. β-adrenoceptor agonist; stereoisomer; stereoselectivity; 1-anthroyl cyanide; chiral HPLC; chiral inversion

It is widely recognized that enantiomers of many chiral drugs exhibit differences in pharmacological and toxicological effects. In addition, recent progress in the development of analytical techniques has revealed considerable differences in the pharmacokinetics and drug disposition of enantiomers. Therefore, it has become fundamentally important to determine the pharmacological, toxicological, and pharmacokinetic profiles of enantiomers.

BRL35135A† (Fig. 1) is a novel antidiabetic and antiobesity agent synthesized by Smith Kline Beecham Pharmaceuticals. It is a potent and selective agonist of β_3-adrenoceptors associated with lipolysis [1–4].

BRL35135A and BRL37344 (Fig. 1), a pharmacologically active metabolite of BRL35135A, are racemic mixtures containing equal amounts of (*RR*) isomer and (*SS*) isomer, and have two independent asymmetric carbons in their molecules. Consequently, there are four possible stereoisomers: (*RR*), (*SS*), (*SR*), and (*RS*).

For the purpose of determining the stereoselectivity of BRL37344 in its pharmacological effects, we evaluated the agonistic effects of each stereoisomer of BRL37344 *in vitro* on β_1-, β_2-, and β_3-adrenoceptors using rat right atria, guinea pig trachea, and rat brown adipocytes, respectively. In addition, to clarify the stereospecific pharmacokinetics of BRL37344, we studied chiral inversion of the compound in rats, determining the plasma concentrations of four stereoisomers of [^{14}C]BRL37344 after oral administration of either [^{14}C](*RR*)BRL35135A or [^{14}C](*SS*)BRL35135A to male rats, by preparing 1-anthroyl cyanide derivatives prior to chiral HPLC.

MATERIALS AND METHODS

Drugs

Racemate (*RR* + *SS*), (*RR*), (*SS*), (*SR*) (sodium salt), and (*RS*)BRL37344 (sodium salt) were synthesized in our labo-

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† *Abbreviations:* BRL35135A, (*R**,*R**)-(±)-methyl-4-[2-[2-hydroxy-2-(3-chlorophenyl)ethylamino]propyl]-phenoxyacetate hydrobromide; BRL37344, (*R**,*R**)-(±)-methyl-4-[2-[2-hydroxy-2-(3-chlorophenyl)-ethylamino]propyl]-phenoxyacetic acid); and RIA, relative intrinsic activity.

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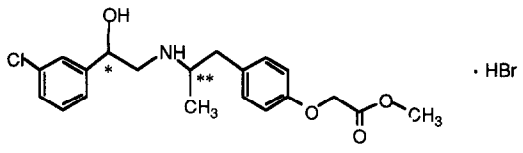
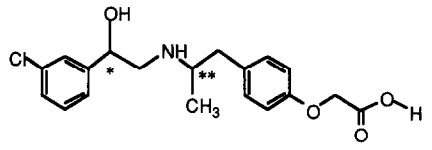


BRL35135A



BRL37344

	*	**	
BRL35135A	R	R	(RR,SS)diastereomer
	S	S	
BRL37344	R	R	(RR,SS)diastereomer
	S	S	
(RR)BRL37344	R	R	
(RS)BRL37344	R	S	
(SR)BRL37344	S	R	
(SS)BRL37344	S	S	

FIG. 1. Chemical structure and stereochemical aspect of BRL37344 and BRL35135A.

ratory. The (*RR* + *SS*) isomer was obtained by hydrolyzation of (*RR* + *SS*)BRL35135A, which was synthesized by Smith Kline Beecham Pharmaceuticals (SKB). Four highly pure stereoisomers of BRL37344 were synthesized by the following procedures from the corresponding crude optically active methyl esters prepared in our laboratory in accordance with the SKB patent [5, 6]. These esters were converted to corresponding cyclic urethane derivatives by carbonyldiimidazole and refined by preparative silica gel chromatography to achieve high purity, in accordance with the patent of American Cyanamid [7]. These cyclic urethane methyl esters were hydrolyzed with sodium hydroxide to obtain highly pure stereoisomers of BRL37344. The enantiomeric purities of these compounds were greater than 97.6% (*RR*), 98.4% (*SS*), 99.0% (*RS*), and 98.5% (*SR*), as determined by chiral HPLC. (±)-Isoprenaline was obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). (*RR* + *SS*)-, (*RR*)-, and (*SS*)BRL37344 were dissolved in a small amount of 1 N NaOH aqueous solution, and diluted with physiological saline. (*RS*)- and (*SR*)BRL37344 were dissolved in physiological saline. (±)-Isoprenaline was dissolved in physiological saline containing 1 mg/mL L-ascorbic acid. [*Chlorobenzene* ring-U-^{14}C](*RR*)BRL35135A ([^{14}C])-(*RR*)BRL35135A) and [*chlorobenzene* ring-U-^{14}C](*SS*)BRL35135A ([^{14}C])-(*SS*)BRL35135A) were separated by Amersham International plc. (Buckinghamshire, England) from [*chlorobenzene* ring-U-^{14}C]racemate, which had been synthesized by Amersham International plc., using a preparative chiral HPLC. these isomers were found by chiral HPLC to have radiochemical purities above 98.2% and to contain less than 1% of other isomers. The specific radioactivities were 11.4 and 10.7 MBq/mg, respectively. For administration, these drugs were dissolved in a small amount of dimethyl sulfoxide and diluted with 0.5% (w/v) carboxymethyl cellulose sodium salt aqueous solution.

Chemicals

Collagenase Type II, BSA (fraction V; fatty acid free) and L-ascorbic acid were obtained from Sigma. 1-Anthroyl cyanide was purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). Acetonitrile (HPLC grade) and methanol (HPLC grade) were from Kokusan Chemical Works, Ltd. (Tokyo, Japan). AQUASOL-2® was obtained from Du Pont/NEN Research Products (Boston, MA, U.S.A.). All other chemicals were of the highest-grade reagent available commercially.

Rat Atrial Rate, β_1-Adrenoceptor-Mediated Response

Male Wistar rats (183–302 g, supplied by Japan SLC, Inc., Shizuoka, Japan), aged 7–10 weeks, were used. They were killed by decapitation and exsanguinated, and right atria were isolated using the method of Broadley and Lumley [8]. The tissues were excised and suspended with a resting tension of 0.5 g in a glass tissue bath containing Krebs-Henseleit buffer solution (NaCl, 118.0 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; $MgSO_4 \cdot 7H_2O$, 1.2 mM; KH_2PO_4, 1.2 mM; $NaHCO_3$, 25.0 mM; glucose, 10.5 mM) bubbled with 5% CO_2 in O_2 at 30°. The heart rate was recorded with a cardiotachometer (AT-600G, Nihon–Koden Kogyo Co., Tokyo, Japan). Spontaneous contraction of the atria was recorded isometrically on an oscillograph using a force-displacement transducer (TB-611T, Nihon Koden) and a carrier amplifier (AP-620G or AP-621G, Nihon Koden). After the rate and amplitude of spontaneous contraction reached steady state, the β-adrenoceptor agonists were added cumulatively to the glass tissue bath. The activity of each agonist was expressed as a percentage of the maximum increase in atrial rate induced by (*RR*)BRL37344. The EC_{50} values were calculated by the probit method as the agonist concentration at half of the maximum response. RIA values were calculated as the ratios of the maximum response of the agonist to the maximum response of (*RR*)BRL37344.

Guinea Pig Trachea Relaxation, β_2-adrenoceptor-Mediated Response

Male Hartley guinea pigs (370–440 g, supplied by Japan SLC, Inc.), aged 4–6 weeks, were used. They were killed by a blow to the head and exsanguinated. Tracheal preparations were prepared using the method of Emmerson and Mackay [9]. The preparations were suspended with a resting tension of 0.5 g in a glass tissue bath containing Tyrode's

solution (NaCl, 137.0 mM; KCl 2.7 mM; $CaCl_2$, 1.8 mM; $NaHCO_3$, 11.9 mM; $NaH_2PO_4 \cdot 2H_2O$, 0.4 mM; $MgCL_2 \cdot 6H_2O$, 1.0 mM; glucose 5.6 mM) bubbled with 5% CO_2 in O_2 at 37°. Tension was recorded isometrically on an oscillograph using a force-displacement transducer and a carrier amplifier. After the tension reached steady state, the β-adrenoceptor agonists were added cumulatively to the glass tissue bath. The activity of each agonist was expressed as a percentage of the maximum relaxation induced by (*RR*)BRL37344. The EC_{50} and RIA values were calculated as for the atrial rate experiments.

Lipolysis of Rat Brown Adipocytes, β_3-Adrenoceptor-Mediated Response

Male Sprague–Dawley rats (270–319 g, supplied by Japan SLC, Inc.), aged 8–9 weeks, were used. They were killed by decapitation and exsanguinated. The adipocytes were prepared essentially in accordance with the method of Rodbell [10]. Briefly, brown fat tissues were removed and chopped into 1-mm slices. The tissues were placed in a polypropylene vessel containing 3 mL of Krebs–Henseleit buffer solution (pH 7.4; NaCl, 118.4 mM; KCl, 4.7 mM; $CaCl_2$, 1.25 mM; $NaHCO_3$, 25 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM; glucose 20 mM) bubbled with 5% CO_2 in O_2, 4% (w/v) BSA, and 1 mg/mL collagenase. Isolation of adipocytes was performed in a shaking waterbath at 37° with 120 strokes/min. After 1 hr, the cells were filtered through nylon mesh (No. 200). Adipocytes were washed three times with Krebs–Henseleit buffer bubbled with 5% CO_2 in O_2, pH 7.4, 37°, containing 4% (w/v) BSA, and incubated in small polypropylene vessels in the same Krebs–Henseleit buffer. Cells were incubated in the presence of different concentrations of BRL37344 or 10^{-6} M isoprenaline for 90 min; then the incubation was terminated by heating at 95° for 10 min. After centrifugation, the upper layer was used for enzymatic glycerol determination. Glycerol assay kits were purchased from Boehringer Mannheim GmbH (Mannheim, Germany). The EC_{50} and RIA values were calculated as described above.

Chiral Inversion in Rat

After oral administration of [^{14}C](*RR*)BRL35135A or [^{14}C](*SS*)BRL35135A at a dose of 1 mg (free base)/kg to male rats, heparinized blood samples were obtained from the femoral vein at 0.5 and 2 hr. Plasma samples were separated after immediate centrifugation of blood samples and stored at −20° until assayed. To determine the stability of (*RR*)BRL37344 in physiological solution, [^{14}C]-(*RR*)BRL37344 was dissolved in 1/15 M phosphate buffer at pH 7.4 and incubated at 37° for 2 hr. Aliquots of the incubated buffer were taken for the same analysis as the plasma. The plasma sample (0.5 or 1 mL) was applied to a Sep-Pak® plus tC_{18} Cartridge (Waters, Milford, MA, U.S.A.) and washed with water (2 mL) and 15% (v/v) methanol. The desired fraction was obtained by elution with 50% (v/v) methanol (2 mL). The eluent was evaporated to dryness, and then 1-anthroyl cyanide in acetonitrile (2 mg/mL, 1 mL) and 10% (v/v) triethylamine in acetonitirile (0.1 mL) were added. The solution was allowed to stand at 60° for 2 hr. After evaporation to dryness, the residue was redissolved in 33% (v/v) acetonitrile in 100 mM phosphate buffer (pH 7.0, 0.4 mL). The solution was applied to a Sep-Pak® light C_{18} Cartridge (Waters) and washed with 33% (v/v) acetonitrile in 100 mM phosphate buffer (pH 7.0, 3 mL) and water (1 mL). The desired fraction was obtained by elution with 50% (v/v) acetonitrile (1 mL). After evaporation of the solvent, the residue was reconstituted in 50% (v/v) acetonitrile (0.2 mL), and a 20-μL aliquot of the solution was injected onto the chiral HPLC after filtration. The apparatus used for this experiment was an 880-PU solvent delivery system (Japan Spectroscopic Co., Ltd., Tokyo, Japan) equipped with a CHIRALCEL® OD-R (4.6 × 250 mm, Daicel Chemical Industries, Ltd., Tokyo, Japan), an 870-UV UV-detector (Japan Spectroscopic Co., Ltd.) and a C-R4AX integrator (Shimazu Co., Kyoto, Japan). The mobile phase consisted of 100 mM phosphate buffer (pH 2.0)-acetonitrile (65:35, v/v). The column was eluted at 0.5 mL/min and its temperature maintained at 30° with monitoring of the eluate with a UV detector (260 nm). An FC-80K fraction collector (Gilson Medical Electronics, Inc., Middleton, WI, U.S.A.) was used to collect 1-min fractions for determination of radioactivity (40 min–100 min). Total radioactivity in the collected fractions was measured after the addition of 10 mL of AQUASOL-2® with an LS6000SC liquid scintillation counter (Beckman Instruments, Inc., Fullerton, CA, U.S.A.). Retention times of the derivatized [^{14}C]BRL37344 isomers were compared with those of the authentic BRL37344 isomers.

RESULTS

Rat Atrial Rate, β_1-Adrenoceptor-Mediated Response

Concentration–response curves for the right atrial rate for the stereoisomers of BRL37344 are shown in Fig. 2, and the EC_{50} values for these compounds are given in Table 1. (*RR* + *SS*)- and (*RR*)BRL37344 induced concentration-dependent increases in atrial rate with EC_{50} values of 5.7×10^{-7} and 2.4×10^{-7} M, respectively. The RIA value of the (*RR* + *SS*) isomer was 1.1. Neither the (*SS*) nor the (*SR*) isomer increased the atrial rate at a concentration up to 10^{-5} M. The increase in atrial rate by the (*RS*) isomer at a concentration of 10^{-5} M was 45% of the maximum effect of the (*RR*) isomer. The maximum effect of (*RR*)BRL37344 was 103% of the maximum effect of (±)-isoprenaline.

Guinea Pig Trachea Relaxation, β_2-Adrenoceptor-Mediated Response

Concentration–response curves for tracheal relaxation for the stereoisomers of BRL37344 are shown in Fig. 3, and the EC_{50} values for these compounds are given in Table 1.

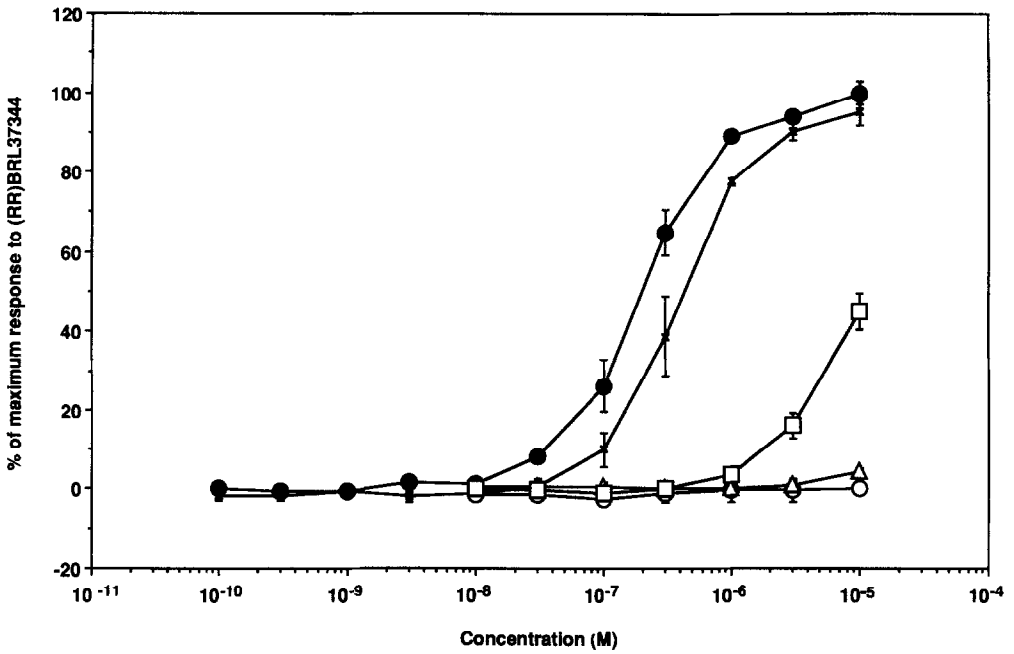


FIG. 2. Concentration–response curves of racemate (*RR* + *SS*) (X), (*RR*) (●), (*SS*) (○), (*SR*) (△), and (*RS*)BRL37344 (□) on the right atrial rate (β_1). Responses are expressed as a percentage of the maximum response to (*RR*)BRL37344. Data are the means ± SEM from three experiments. The maximum effect of (*RR*)BRL37344 was 103% of the maximum effect of (±)-isoprenaline.

These isomers induced concentration-dependent relaxation of guinea pig trachea. Half-maximal stimulation by the (*RR* + *SS*) and (*RR*) isomers occurred at 1.8×10^{-8} M and 8.0×10^{-9} M, respectively. The RIA value of the (*RR* + *SS*) isomer was 1.0. The (*SS*), (*SR*), and (*RS*) isomers were much less potent, and their effects at 10^{-5} M were 35, 77 and 82% of the maximum effect of the (*RR*) isomer, respectively. The maximum effect of (*RR*)BRL37344 was 99% of the maximum effect of (±)-isoprenaline.

Lipolysis of Rat Brown Adipocyte, β_3-Adrenoceptor-Mediated Response

Concentration–response curves for lipolysis of rat brown adipocytes for the stereoisomers of BRL37344 are shown in Fig. 4, and the EC_{50} values for these compounds are given in Table 1. (*RR* + *SS*)-, (*RR*)- and (*SR*)BRL37344 induced concentration-dependent lipolysis of brown adipocytes with EC_{50} values of 5.3×10^{-9}, 2.6×10^{-9} and 2.5×10^{-6} M, respectively. The RIA values of (*RR* + *SS*) and (*SR*) were 0.98 and 1.03. Stimulation of adipocyte lipolysis by the (*SS*) isomer at a concentration of 10^{-6} M and by the (*RS*) isomer at a concentration of 10^{-4} M was 30 and 88% of the maximum effect of the (*RR*) isomer, respectively. The maximum effect of (*RR*)BRL37344 was 97% of the maximum effect of (±)-isoprenaline.

Chiral Inversion in the Rat

Table 2 shows the plasma levels of radioactivity in male rats at 0.5 and 2 hr after oral administration of either [^{14}C](*RR*)BRL35135A or [^{14}C](*SS*)BRL35135A at a dose of 1 mg (free base)/kg. Figure 5 shows the chiral HPLC profiles of the optical isomers of BRL37344 after oral administration of [^{14}C](*RR*)BRL35135A to male rats. At 2 hr, (*SR*) BRL37344 was found with the (*RR*) isomer, the ratio of peak areas (*SR*/*RR*) being 0.48 ± 0.11 (mean ± SD). However, when [^{14}C](*SS*)BRL35135A was administered, as

TABLE 1. Agonist activities of racemate or optical isomers of BRL37344 for β-adrenoceptor subtypes

	Brown adipocyte lipolysis (β_3)		Right atrial rate (β_1)		Tracheal relaxation (β_2)		Selectivity for lipolysis over:	
Agonist	EC_{50} (nM)	Ratio	EC_{50} (nM)	Ratio	EC_{50} (nM)	Ratio	Atria	Trachea
(*RR* + *SS*)BRL37344	5.3	2.0	570	2.4	18	2.3	110	3.4
(*RR*)BRL37344	2.6	1.0	240	1.0	8.0	1.0	92	3.1
(*SS*)BRL37344	>1,000	>380	>10,000	>420	>10,000	>1,300		
(*SR*)BRL37344	2,500	960	>10,000	>420	>1,000	>130		
(*RS*)BRL37344	>300	>120	>10,000	>420	>300	>38		

EC_{50}: molar EC_{50} values are expressed relative to the maximal effect of (*RR*)BRL37344. Ratio: molar EC_{50} value of the agonist/molar EC_{50} value of (*RR*)BRL37344. Selectivity value: molar EC_{50} value of the agonist for atria or trachea/molar EC_{50} value of the agonist for lypolysis.

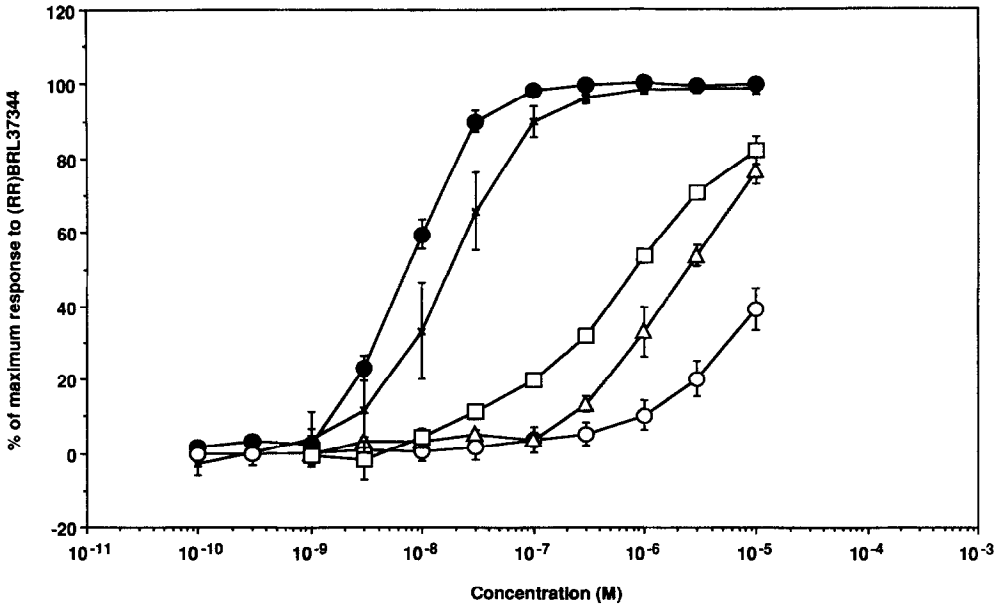


FIG. 3. Concentration–response curves of racemate (*RR* + *SS*) (X), (*RR*) (●), (*SS*) (○), (*SR*) (△), and (*RS*)BRL37344 (□) on tracheal relaxation (β_2). Responses are expressed as a percentage of the maximum response to (*RR*)BRL37344. Data are the means ± SEM from three experiments. The maximum effect of (*RR*)BRL37344 was 99% of the maximum effect of (±)-isoprenaline.

shown in Fig. 6, no other isomers except (*SS*)BRL37344 were detected at 0.5 and 2 hr. When [^{14}C](*RR*)BRL37344 was incubated in phosphate buffer (pH 7.4) at 37° for 2 hr, racemization of the (*RR*) isomer did not occur.

DISCUSSION

Many β-adrenoceptor agonists, including salbutamol [11,12] and terbutaline [13], exhibit stereoselectivity in their effects on adrenoceptors. In the present study, we showed that the agonist effects of the (*SS*), (*SR*), and (*RS*) isomers of BRL37344, which is known to be a β_3-adrenoceptor selective agonist and thermogenic agent [1–4], on β_1-, β_2-, and β_3-adrenoceptor-mediated activities were quite low compared with those of (*RR*)BRL37344, and that BRL37344 exhibits significant stereoselectivity in its effect on β-adrenoceptors, as in the case for other β-adrenoceptor agonists. In a previous study, it was reported that

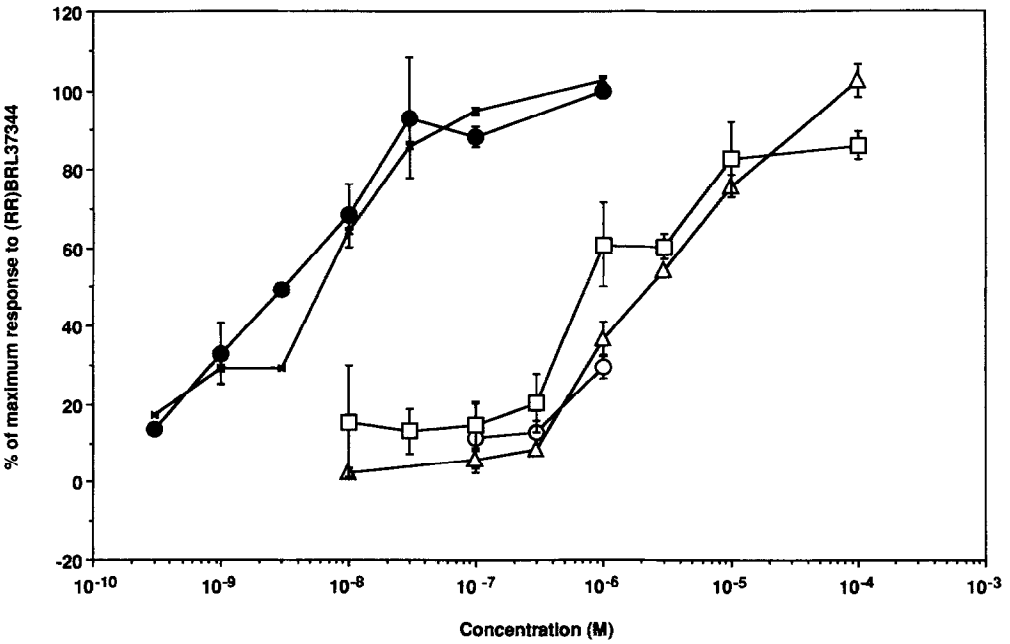


FIG. 4. Concentration–response curves of racemate (*RR* + *SS*) (X), (*RR*) (●), (*SS*) (○), (*SR*) (△), and (*RS*)BRL37344 (□) on brown adipocyte lipolysis (β_3). Responses are expressed as a percentage of the maximum response to (*RR*)BRL37344. Data are the means ± SEM from three experiments. The maximum effect of (*RR*)BRL37344 was 97% of the maximum effect of (±)-isoprenaline.

TABLE 2. Plasma concentration of radioactivity after oral administration of [^{14}C](*RR*)- or [^{14}C](*SS*)BRL35135A to male rats (dose 1 mg (free base/kg)

	Time (hr)	Concentration (ng eq. of BRL35135 free base/mL)
RR	0.5	249.80 ± 32.00
	2.0	92.80 ± 14.48
SS	0.5	473.77 ± 41.32
	2.0	32.61 ± 9.98

Data are expressed as the mean values ± SD for three animals.

the relative potency of the effect of (*RR* + SS)BRL37344 on brown adipocyte lipolysis in rats was 400 and 20.5 times higher than for the right atrial rate in rats and relaxation of trachea in guinea pigs, respectively [1]. However, in the present study, the relative potency of the effect of (*RR* + SS)BRL37344 on brown adipocyte lipolysis was 110 and 3.4 times higher than for the atrial rate and relaxation of trachea, respectively. The comparatively poor selectivity of (*RR* + SS) for the β_2-adrenoceptor cannot be attributed to the presence of the (SS) isomer, which exhibits essentially

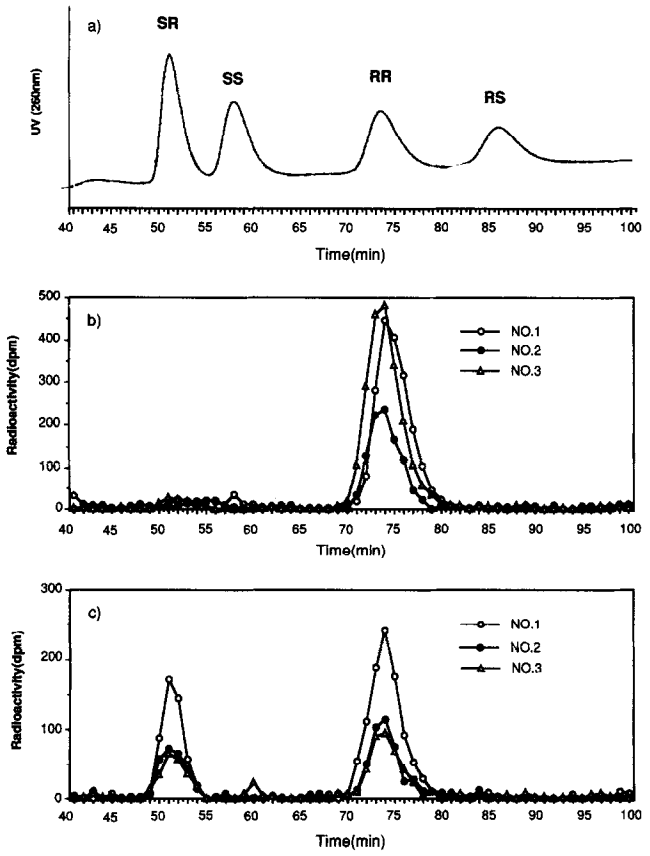


FIG. 5. HPLC analysis of optical isomers of BRL37344 in rat plasma after oral administration of [^{14}C](*RR*)BRL35135A to three male rats (dose 1 mg/kg). Each line represents the individual result obtained from three independent experiments with three male rats. (a) Authentic standard mixture of (RR)-, (SS)-, (RS)- and (RS)BRL37344. (b) Plasma 0.5 hr after oral administration of [^{14}C](*RR*)BRL35135A. (c) Plasma 2 hr after oral administration of [^{14}C]-(*RR*)BRL35135A.

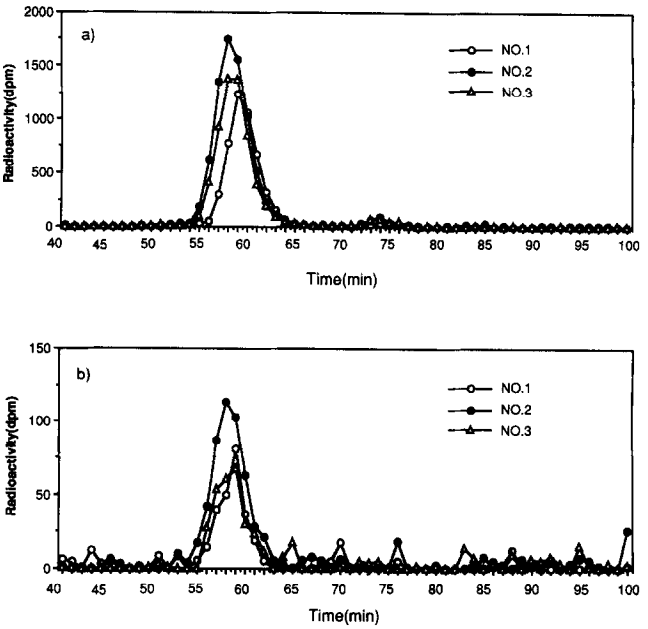


FIG. 6. HPLC analysis of optical isomers of BRL37344 in rat plasma after oral administration of [^{14}C](SS)BRL35135A to three male rats (dose 1 mg/kg). Each line represents the individual result obtained from three independent experiments with three male rats. (a) Plasma 0.5 hr after oral administration of [^{14}C](SS)BRL35135A. (b) Plasma 2 hr after oral administration of [^{14}C](SS)BRL35135A.

no agonist activity up to 10^{-6} M. In another experiment, we examined the β_2-adrenoceptor activity of (*RR* + SS) using rat uterus smooth muscle, and found that (*RR* + SS) exhibited 56 times less potency than β_3-adrenoceptor. In addition, the (SS) isomer did not appear to antagonize the activity of the (*RR*) isomer, since (*RR* + SS) was half as potent as the (*RR*) isomer in all specimens tested.

In HPLC analysis, sufficient separation of the four isome stereoisomers could not be achieved using a chiral HPLC column alone. The derivatization of the hydroxy group with 1-anthroyl cyanide prior to CHIRALCEL® OD-R column chromatography resulted in a good separation of these isomers. The control plasma samples spiked with [^{14}C](*RR* + SS)BRL37344 yielded a low recovery of radioactivity, but the recoveries of the isomers were nearly equal, and no chiral inversion was observed in the process of analysis. We therefore concluded that the chiral inversion of BRL37344 *in vivo* could be investigated using this improved method. The mean level of plasma radioactivity in rats 0.5 hr after the oral administration of [^{14}C](SS)BRL37344A was 1.9 times as high as that with administration of [^{14}C](*RR*)-BRL35135A. At 2 hr, however, the mean level of plasma radioactivity in rats administered [^{14}C](*RR*)BRL35135A was 2.8 times as high as that with [^{14}C](SS)BRL35135A. This finding suggests that there is stereoselectivity in any phase of absorption and disposition of BRL35135A. Moreover, chiral inversion of [^{14}C](*RR*)BRL37344 to the (*SR*) isomer was observed, but inversion of [^{14}C](SS)BRL37344 was not. In a control experiment, (*RR*)BRL37344 was not converted to the (*SR*) isomer in physiological phosphate buffer. This indicates that stereoselective chiral inversion of

BRL37344 occurs enzymatically in rats. Enzymatic dehydrogenation of hydroxy compounds to carbonyl compounds and rehydrogenation of the carbonyl compounds so formed may occur in both animals and humans. It has been demonstrated that 3-hydroxyhexobarbital dehydrogenase from guinea pig and rabbit liver exhibits quite different activity toward the four stereoisomers of 3-hydroxyhexobarbital, and that the configuration of the hydroxy group is a factor significantly affecting the reactivity of the enzyme [14]. In addition, the Baumann–Prelog rule states that carbonyls are preferentially reduced to the corresponding alcohols with *S*-configuration in cytosol fractions of animal and human tissues [15]. In accordance with this rule, stereoselective reduction of carbonyls has been observed for some drugs [16–18]. E2001, a monoamine oxidase inhibitor that contains a stereogenic center with a secondary alcohol group like that of BRL37344, exhibits chiral inversion through a carbonyl intermediate in a number of animal species [19]. The mechanism of the chiral inversion of (*RR*)BRL37344 to (*SR*)BRL37344 observed in the present study has not been investigated, but the observed high stereoselectivity between (*RR*) and (*SS*) isomers and the findings noted above suggest the following hypothesis. The observed inversion of (*RR*)BRL37344 to the (*SR*) isomer may result from a redox reaction, in which either oxidation of the hydroxy group or reduction of the ketone (or both reactions) exhibits stereoselectivity such that (*RR*)BRL37344 is converted to the (*SR*) isomer, without conversion of the (*SS*) isomer.

Although stereoselective chiral inversion of BRL37344 from the (*RR*) isomer to the (*SR*) isomer has been observed in rats, *in vivo* pharmacological effects of other isomers may be left out of the consideration, since (*SS*), (*SR*), and (*RS* isomers have very low activities for β-adrenoceptors compared with the (*RR*) isomer. However, it has also been suggested that the *in vivo* pharmacological effect of BRL35135A may be less potent than that expected from its *in vitro* agonist activity. The findings of the present study suggest that quantitative determination of the pharmacologically active (*RR*)BRL37344 in biological fluids is of importance to complete understanding of its *in vivo* pharmacological effect.

Previous studies on the *in vitro* side chain carbonyl reduction of warfarin and its analogues have demonstrated that in microsomal fractions, large differences in alcohol formation rates as well as substrate and product stereoselectivity are observed between species [18]. In addition, stereoselectivity has been observed in all phases of drug absorption and disposition [20]. Consequently, the pharmacokinetics of the stereoisomers of BRL37344 should be studied to determine the pharmacological and toxicological effects of this agent in animals and humans.

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