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Improvement of Metabolic Disorders and Visceral Fat Obesity by the β_3-Adrenoceptor Agonist (R^*,R^*)-(±)-Methyl-4-[2-[2-hydroxy-2-(3-chlorophenyl)ethylamino]propyl]-phenoxyacetate Hydrobromide (BRL35135A) in Genetically Obese Rodents

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ABSTRACT. The effects of BRL35135A ((R^*,R^*)-(±)-methyl-4-[2-[2-hydroxy-2-(3-chlorophenyl)ethylamino]propyl]-phenoxyacetate hydrobromide), a β_3-adrenoceptor agonist, on visceral and subcutaneous fat weight and metabolic disorders were studied in genetically obese C57BL/KsJ db/db mice and Zucker fa/fa rats. In db/db mice, four weeks of oral administration of BRL35135A (0.5 and 5 mg/kg/day) decreased body weight gain and reduced white fat weight. The rates of reduction of white fat weight were in the order mesenteric fat > retroperitoneal fat > subcutaneous fat. In fa/fa rats, daily administration of BRL35135A (0.05 mg/kg/day)) for 6 weeks reduced the visceral white fat weight/total energy intake ratio, particularly for mesenteric fat, without any clear effect on body weight gain. This tendency of the compound to exert effects on visceral fat was consistent with the findings that the effect of BRL37344 ((R^*,R^*)-(±)-methyl-4-[2-[2-hydroxy-2-(3-chlorophenyl)ethylamino]propyl]-phenoxyacetic acid), an active metabolite of BRL35135A, on the lipolytic activity of isolated adipocytes and the tissue concentration of [^{14}C]BRL37344 in male Wistar rats were each greater in visceral fat than in subcutaneous fat. Moreover, BRL35135A at 0.05 mg/kg/day elevated serum insulin levels and improved hyperglycemia in db/db mice without reducing body weight gain, whereas at doses of 0.5 and 5 mg/kg/day it ameliorated hyperglycemia and hyperlipidemia, and tended to decrease serum insulin levels. In fa/fa rats, BRL35135A (0.005 mg/kg/day) was also effective in improving hyperinsulinemia, glucose intolerance, and hypertriglyceridemia without any effect on body weight gain or fat distribution. These findings suggest that the improvement of metabolic disorders by BRL35135A may be due to improvement in insulin resistance as well as reduction of visceral fat weight. BIOCHEM PHARMACOL 52;10:1529–1535, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. β_3-adrenoceptor agonist; body weight; visceral fat; insulin resistance; lipolysis; genetically obese rodents

Obesity is associated with a high incidence of metabolic disorders including glucose intolerance, hyperinsulinemia, and hyperlipidemia. Recent studies have demonstrated that these obesity-related metabolic disorders are strongly affected by the regional distribution of fat. In particular, visceral fat obesity more frequently accompanies these metabolic disorders than does subcutaneous fat obesity [1, 2]. The metabolic characteristics of visceral fat are known to be more sensitive to lipolytic agents, such as β-adrenoceptor agonists, than are those of subcutaneous fat [3, 4].

Recently, β_3-adrenoceptors have been shown to mediate lipolysis in white and brown adipocytes [5, 6], and the β_3-adrenoceptor gene has been cloned from human, rat, and mouse genomic or cDNA libraries and characterized [7–10]. Since the finding of the β_3-adrenoceptor, several β_3-adrenoceptor agonists [11–13], including BRL35135A† [5], have been found. These agents stimulate lipolysis in white and brown adipocytes, and chronic treatment with them results in antiobese effects due to enhancement of thermogenesis in brown fat. They also improve metabolic disorders such as hyperglycemia, hyperlipidemia, and hyperinsu-

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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 OGTT, oral glucose tolerance test.

linemia in genetically obese rodents. However, the reasons for the improvement of visceral fat obesity and metabolic disorders by β_3-adrenoceptor agonists remain unknown.

In the present studies, we investigated the effect of BRL35135A on fat distribution, and attempted to clarify how it improves metabolic disorders in genetically obese and hyperinsulinemic C57BL/KsJ db/db mice and Zucker fa/fa rats. These rodents are models of human obese non-insulin-dependent diabetes mellitus (NIDDM); the former exhibit extreme hyperglycemia, whereas the latter exhibit glucose intolerance and extreme hypertriglyceridemia. In addition, we studied the tissue distribution and lipolytic activity of BRL37344, an active metabolite of BRL35135A, to clarify the difference in the effects of BRL35135A on visceral and subcutaneous fat.

MATERIALS AND METHODS

Drugs

BRL35135A was synthesized by Smith Kline Beecham Pharmaceuticals (Middlesex, U.K.). BRL37344 was obtained by hydrolysis from BRL35135A in our laboratory. [*Chlorobenzene* ring-U-^{14}C]BRL35135A was purchased from Amersham International plc. (Buckinghamshire, U.K.); its specific radioactivity was 9.66 MBq/mg, and its radiochemical purity was 98% was determined by HPLC. (±)-Isoprenaline was obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Chemicals

Collagenase (Type II) and BSA (fraction V; fatty acid-free) were from Sigma. ATOMLITE® and SOLVABLE® were obtained from Du Pont/NEN Research Products (Boston, MA, U.S.A.). All other chemicals were of the highest grade available commercially.

Antiobesity and Antidiabetic Study of C57BL/KsJ db/db Mice

Female C57BL/KsJ db/db mice (36.6 to 50.3 g) and their lean db/+ littermates (22.1 to 23.4 g) were purchased from Charles River Japan, Inc. (Kanagawa, Japan) and were used at 9 weeks of age. Db/db mice were divided into four groups of eight each; neither body weight nor serum glucose level differed significantly among the groups. Eight lean littermates were also used. The mice were maintained on laboratory chow [CE-2: 14.2 kJ/g, Clea Japan, Inc. (Tokyo, Japan)] and water *ad lib.*, and were housed in a temperature- and humidity-controlled room (22 ± 1°, 50 ± 5%) with lighting from 8:00 a.m. to 8:00 p.m. Body weight was measured daily, and the intake of food and water was measured at 2- or 3-day intervals. BRL35135A was dissolved in distilled water and administered orally once daily for 4 weeks at doses of 0.05, 0.5, or 5 mg/kg/day. Control mice were administered vehicle alone. The lean littermates were given no treatment. The day after the last administration, mice were killed by decapitation, and blood samples were collected; then mesenteric fat, retroperitoneal fat, and subcutaneous fat were removed and weighed. Plasma glucose, free fatty acid, triglyceride, and total cholesterol were measured using colorimetric kits (based on glucose oxidase, acyl-coA oxidase, glycerol-3-phosphate oxidase, and cholesterol oxidase methods, respectively) purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Serum insulin was determined using an enzyme immunoassay kit from the Sanko Junyaku Co., Ltd. (Tokyo, Japan).

Antiobesity and Antidiabetic Study of Zucker fa/fa Rats

Female Zucker fa/fa rats (310–390 g) and their lean fa/+ littermates (130–220 g) were purchased from Charles River Japan, Inc., were used at 10 weeks of age, and were kept as described above. Zucker fa/fa rats were divided into four groups of six in such a fashion that neither body weight nor serum glucose level differed significantly among the groups. Six lean littermates were also used. BRL35135A was dissolved in distilled water and administered orally once daily for 6 weeks at doses of 0.005, 0.015, or 0.05 mg/kg/day. Control rats were administered vehicle alone. The lean littermates were not given any treatment. One-half hour after the first administration, heart rate was measured with a programmable sphygmomanometer (PS-600 Riken Kaihatsu, Tokyo, Japan) in conscious fa/fa rats. Five hours before the last administration, blood samples were obtained from the retro-orbital sinus, and serum glucose, insulin, free fatty acid, triglyceride, and total cholesterol were measured. Serum triglyceride and free fatty acid were assayed using colorimetric kits based on the acetylacetone and modified Duncombe methods, respectively; the other parameters were assayed using the same methods as for db/db mice. The OGTT was performed at the last administration in 5-hr fasted fa/fa rats and their lean littermates. One-half hour after the administration of BRL35135A, glucose at a dose of 2 g/kg was administered orally. Blood samples were taken from the jugular vein of ether-anesthetized rats just before and at 30, 60, and 120 min after oral glucose loading, and plasma glucose levels were determined. Plasma obtained 0.5 hr after the last administration of BRL35135A was used for the measurement of potassium levels, which were determined with an autoelectrolyte analyzer (SERA-520; Horiba Industries, Ltd., Kyoto, Japan). After the OGTT, rats were killed by decapitation, and mesenteric, retroperitoneal, and subcutaneous fat removed and weighed.

Lipolysis Study

Male Sprague–Dawley rats, supplied by Japan SLC, Inc. (Shizuoka, Japan), 7 or 8 weeks of age, 248–308 g, were used. The rats were kept as described above. Isolation of adipocytes was carried out essentially in accordance with the method of Rodbell [14]. The rats were killed by decapitation, and then mesenteric, perirenal, and subcutaneous fat was immediately removed and chopped into approxi-

mately 1-mm slices. The chopped tissue (3 g) was placed in a polypropylene vessel containing 9 mL of Krebs–Henseleit solution (NaCl, 118.4 mM; KCl, 4.7 mM: $CaCl_2 \cdot 2H_2O$, 1.25 mM; $NaHCO_3$, 25 mM; $MgSO_4 \cdot 7H_2O$, 1.2 mM; $KH_2PO_4 \cdot 2H_2O$, 1.2 mM; glucose, 20 mM; pregassed with 95% O2–5% CO_2, pH 7.4) containing 4% (w/v) BSA and 1 mg/mL collagenase, and adipocytes were prepared by shaking (120 strokes/min) at 37° for 60 min. Then the cells were filtered through nylon mesh (No. 200) and washed three times with Krebs–Henseleit buffer pregassed with 95% O_2–5% CO_2, 37°, containing 4% (w/v) BSA (fatty acid-free), and incubated in small polypropylene vessels in the same Krebs–Henseleit buffer. The cells (5×10^5 cells/mL) were incubated in the presence of various concentrations of BRL37344 and 10^{-6} M isoprenaline for 90 min; 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} values were expressed relative to the maximal effect of BRL37344. Relative intrinsic activities were calculated as follows: maximal effect of BRL37344/maximal effect of isoprenaline (10^{-6} M).

Tissue Distribution Study

Male Wistar rats, supplied by Japan SLC Inc., 8 weeks of age, 183–194 g, were fasted for 16 hr before drug administration. They were kept as described above. [*Chlorobenzene* ring U-^{14}C]BRL35135A, in the present experiment, was dissolved in distilled water, and then diluted with 0.5% carboxymethyl cellulose aq. [^{14}C]BRL35135A was given orally at a dose of 1 mg/kg. One-half hour after administration, rats were anesthetized with ether, blood was collected from the jugular vein, and the rats were killed by exsanguination via the carotid artery. Then plasma, mesenteric fat, perirenal fat, subcutaneous fat, and intracapsular brown fat were dissolved in SOLVABLE®. These solutions were diluted by ATOMLITE®, and radioactivity was measured with a liquid scintillation counter (LS 6000 SC; Beckman Instruments, Inc., Fullerton, CA, U.S.A.).

Statistical Analysis

Values are expressed as means ± S.E.M. Statistical analysis was performed using one-way ANOVA followed by the Bonferroni test or Student's *t*-test.

RESULTS

Effects on C57BL/KsJ db/db Mice

Vehicle-treated C57BL/KsJ db/db mice were obese, with an increase in visceral and subcutaneous white fat weight compared with those of lean mice (Figs. 1 and 2). The final mean white fat weights and body weight in vehicle-treated db/db mice were as follows: subcutaneous fat 12.4 ± 0.3 g, retroperitoneal 5.8 ± 0.2 g, mesenteric 2.0 ± 0.1 g, and body weight 52.6 ± 0.6 g. Administration of BRL35135A at a dose of 0.05 mg/kg/day for 4 weeks affected neither body weight gain nor visceral or subcutaneous white fat weight (Figs. 1 and 2). However, at doses of 0.5 and 5 mg/kg/day, BRL35135A decreased body weight gain beginning at 4 and 2 weeks, respectively; this effect was greater at 5 mg/kg/day than at 0.5 mg/kg/day (Fig. 1). In addition to body weight

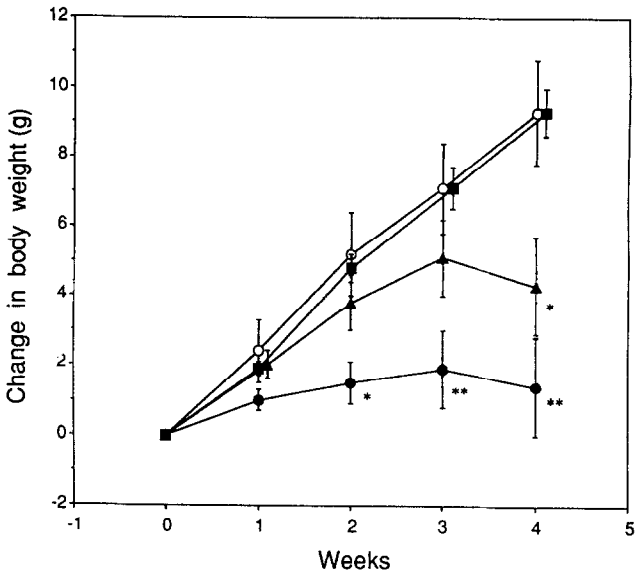

FIG. 1. Effect of BRL35135A on body weight change in genetically obese C57BL/KsJ db/db mice. There were eight mice in each group. Values are means ± SEM at each point for vehicle (○), and BRL35135A at 0.05 mg/kg/day (■), 0.5 mg/kg/day (▲) and 5 mg/kg/day (●). Key: (*)$P < 0.05$ and (**)$P < 0.01$, vs vehicle-treated mice (Bonferroni test).

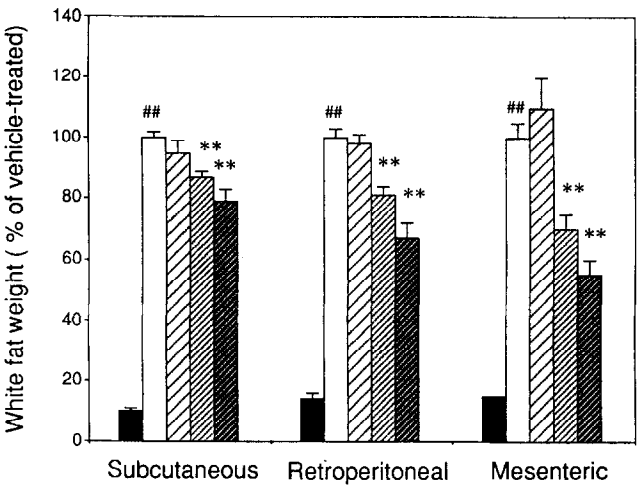

FIG. 2. Rate of fat weight reduction induced by BRL35135A in genetically obese C57BL/KsJ db/db mice. There were eight mice in each group. Values are means ± SEM for eight mice. The percentages of values of vehicle-treated db/db mice are given in the text. Key: Lean (■), vehicle (□), and BRL35135A at 0.05 mg/kg/day (▨), 0.5 mg/kg/day (▨), 5 mg/kg/day (▩). The mean white fat weights in vehicle-treated db/db mice were as follows: subcutaneous fat 12.4 ± 0.3 g; retroperitoneal, 5.8 ± 0.2; and mesenteric, 2.0 ± 0.1. Key: (**)$P < 0.01$, vs vehicle-treated mice (Bonferroni test), and (##)$P < 0.01$, vs lean db/+ mice (Student *t*-test).

gain, visceral and subcutaneous white fat weights were diminished in a dose-dependent fashion by BRL35135A at doses of 0.5 and 5 mg/kg/day (Fig. 2). The order, by percentage of fat weight in vehicle-treated mice, was: subcutaneous fat (79% at a dose of 5 mg/kg/day) > retroperitoneal fat (67%) > mesenteric fat (57%) (Fig. 2). Total energy intakes were decreased slightly, to the same extent at each BRL35135A dose (Table 1). Total water intake during week 4 was as follows: control, 15.6 (mL/day); BRL35135A 0.05 mg/kg/day, 6.5; BRL35135A 0.5 mg/kg/day, 5.1; and BRL35135A 5 mg/kg/day, 5.9.

C57BL/KsJ db/db mice exhibited metabolic disorders including hyperglycemia, hyperinsulinemia, and hyperlipidemia in comparison with the lean mice (Table 1). BRL35135A at a dose of 0.05 mg/kg/day improved hyperglycemia moderately, and at doses of 0.5 and 5 mg/kg/day normalized hyperglycemia. Serum insulin levels were increased significantly by BRL35135A at a dose of 0.05 mg/kg/day, but were lowered slightly below the levels in vehicle-treated mice at doses of 0.5 and 5 mg/kg/day. BRL35135A at a dose of 0.05 mg/kg/day slightly but not significantly decreased free fatty acid and triglyceride levels. On the other hand, total cholesterol levels were not affected by a dose of 0.05 mg/kg/day. Doses of 0.5 and 5 mg/kg/day decreased the extent of increase in free fatty acid, triglyceride, and total cholesterol (Table 1).

Effect on Zucker fa/fa Rats

Vehicle-treated Zucker fa/fa rats were obese, displaying an increase in visceral and subcutaneous fat weight when compared with lean rats (Table 2). BRL35135A at a dose of 0.05 mg/kg/day did not affect body weight gain or subcutaneous fat weight, but did reduce visceral fat weight slightly. The rates of reduction of visceral white fat weight were greater for mesenteric fat than for retroperitoneal fat. Total energy intake was increased slightly by BRL35135A and in a dose-dependent fashion (Table 2). The (fat weight/total energy intake) ratio was decreased significantly for visceral fat, but not for subcutaneous fat (Table 2). Total water intake during week 6 was as follows: control 25 (mL/day); BRL35135A 0.05 mg/kg/day, 29; BRL35135A 0.5 mg/kg/day, 33; and BRL35135A 5 mg/kg/day, 37.

Serum insulin levels were lowered to the same extent at each dose. BRL35135A improved glucose intolerance at a dose of 0.005 mg/kg/day to below the levels in lean rats. Triglyceride levels were diminished slightly at a dose of 0.005 mg/kg/day, and were decreased dose dependently and significantly at doses of 0.015 and 0.05 mg/kg/day. Neither free fatty acid nor total cholesterol levels were affected by a dose of 0.005 mg/kg/day, but at doses of 0.015 and 0.05 mg/kg/day the former were normalized and the latter were decreased dose dependently. Administration of BRL35135A affected neither heart rate nor serum potassium (Table 3).

Lipolytic Activity of BRL37344

BRL37344 induced dose-dependent lipolysis in rat mesenteric, perirenal, and subcutaneous adipocytes. The EC_{50} values were 11, 34, and 250 nM, and relative intrinsic activities (10^{-6} M isoprenaline = 1.0) were 0.94, 1.04 and 0.84 for rat mesenteric, perirenal, and subcutaneous adipocytes, respectively (Fig. 3).

Tissue Distribution of BRL37344

The tissue concentrations of [^{14}C]BRL37344 at 0.5 hr after oral administration of [^{14}C]BRL35135A (1 mg/kg) to nor-

TABLE 1. Effects of BRL35135A on energy intake, serum glucose, serum insulin, and lipids levels in genetically obese C57BL/KsJ db/db mice

	Lean db/+ mice	db/db Mice			
			BRL35135A		
	Untreated	Vehicle	0.05 mg/kg	0.5 mg/kg	5 mg/kg
Total energy intake (MJ)	1.10 ± 0.03 (44)	2.51 ± 0.13* (100)	2.30 ± 0.13 (92)	2.30 ± 0.06 (92)	2.31 ± 0.02 (92)
Glucose (mg/dl)	157 ± 9 (29)	544 ± 17* (100)	339 ± 50† (62)	172 ± 15† (32)	149 ± 9† (28)
Insulin (μU/mL)	17 ± 2 (17)	99 ± 30* (100)	189 ± 24‡ (190)	86 ± 16 (87)	75 ± 13 (76)
Free fatty acid (μEq/L)	1742 ± 152 (54)	3234 ± 368* (100)	2554 ± 244 (79)	2237 ± 268‡ (69)	2125 ± 158‡ (66)
Triglyceride (mg/dL)	110 ± 8 (34)	321 ± 52* (100)	243 ± 26 (76)	219 ± 19 (68)	182 ± 13‡ (57)
Total cholesterol (mg/dL)	123 ± 9 (46)	265 ± 8* (100)	260 ± 12 (98)	206 ± 3† (78)	235 ± 9‡ (89)

BRL35135A was given p.o. daily for 4 weeks. Total energy intake was calculated from the total food intake for 4 weeks and the energy in the chow (CE-2, 14.2 kJ/g). There were eight mice in each group. Values are means ± SEM for eight mice, and percentages of values for vehicle-treated db/db mice are given in parentheses.

* $P < 0.01$, vs lean db/+ mice (Student's t-test).

† $P < 0.01$, vs vehicle-treated mice (Bonferroni test).

‡ $P < 0.05$, vs vehicle-treated mice (Bonferroni test).

TABLE 2. Effects of BRL35135A on energy intake, body weight gain, and white fat weight in genetically obese Zucker fa/fa rats

	Lean fa/+ rats	fa/fa Rats			
			BRL35135A		
	Untreated	Vehicle	0.005 mg/kg	0.015 mg/kg	0.05 mg/kg
Total energy intake (MJ)	9.60 ± 0.5 (62)	15.5 ± 0.5* (100)	15.7 ± 0.4 (101)	16.2 ± 0.5 (105)	16.9 ± 0.6 (109)
Body weight gain (g)	40 ± 5 (28)	142 ± 9* (100)	136 ± 4 (96)	142 ± 8 (100)	130 ± 8 (92)
Body weight gain/Total energy intake (g/MJ)	4.1 ± 0.4 (45)	9.1 ± 0.3* (100)	8.7 ± 0.1 (95)	8.8 ± 0.4 (96)	7.7 ± 0.4† (84)
Fat weight (g)					
Subcutaneous	4.72 ± 0.9 (5)	96.9 ± 5.5* (100)	94.2 ± 3.7 (97)	92.3 ± 3.8 (95)	92.7 ± 4.1 (96)
Retroperitoneal	5.8 ± 1.2 (11)	53.3 ± 2.6* (100)	52.5 ± 1.6 (99)	52.3 ± 2.3 (98)	47.0 ± 2.5 (88)
Mesenteric	2.2 ± 0.5 (15)	14.4 ± 0.6* (100)	13.5 ± 1.0 (94)	13.2 ± 0.3 (92)	12.1 ± 0.3 (85)
Fat weight/Total energy intake (g/MJ)					
Subcutaneous	0.48 ± 0.08 (8)	6.25 ± 0.27* (100)	5.99 ± 0.13 (96)	5.73 ± 0.33 (92)	5.48 ± 0.13 (88)
Retroperitoneal	0.58 ± 0.11 (17)	3.46 ± 0.21* (100)	3.35 ± 0.10 (97)	3.24 ± 0.13 (94)	2.79 ± 0.12‡ (81)
Mesenteric	0.22 ± 0.04 (24)	0.93 ± 0.04* (100)	0.86 ± 0.06 (92)	0.82 ± 0.04 (88)	0.72 ± 0.02† (77)

BRL35135A was given p.o. daily for 6 weeks. Total energy intake was calculated from the total food intake for 6 weeks and the energy in the chow (CE-2, 14.2 kJ/g). There were six rats in each group. Values are means ± SEM for six rats, and percentages of values for vehicle-treated fa/fa rats are given in parentheses.

* $P < 0.01$, vs lean fa/+ rats (Student's t-test).

† $P < 0.01$, vs vehicle-treated rats (Bonferroni test).

‡ $P < 0.05$, vs vehicle-treated rats (Bonferroni test).

mal rats were in the following order: mesenteric fat > brown fat > perirenal fat > subcutaneous fat (Table 4). The concentration of [^{14}C]BRL37344 in mesenteric fat was almost the same as that in plasma, and was much higher than in the other fat samples.

DISCUSSION

The present study showed that BRL35135A, a potent and selective β_3-adrenoceptor agonist [5], reduced body weight gain and also visceral and subcutaneous white fat weight in genetically obese C57BL/KsJ db/db mice, an animal model with hyperglycemia and severe insulin resistance. The rates of reduction of white fat weight were greater for visceral fat than for subcutaneous fat. This characteristic was exhibited but not to a significant extent, with chronic treatment with BRL35135A at a dose of 0.05 mg/kg in genetically obese Zucker fa/fa rats, an animal model of glucose intolerance, hyperinsulinemia, and hyperlipidemia.

The lipolytic activity of β-adrenoceptor agonists has been shown to be greater in visceral than in subcutaneous fat [3, 4]. This finding was obtained for BRL37344, an active metabolite of BRL35135A, in this study. In addition, the tissue concentration of BRL37344 after oral administration of BRL35135A was in the following order: mesenteric fat > perirenal fat > subcutaneous fat. These findings suggest that both the lipolytic activity of BRL37344 and its tissue concentration are affected by the selective reduction in visceral fat reduction by BRL35135A in obese rodents. β_3-Adrenoceptor content may be associated with these observations, for it has been demonstrated that β_3-adrenoceptor mRNA is more abundant in visceral than in subcutaneous fat [15].

When BRL35135A reduced body weight gain, there was no corresponding significant decrease in total energy intake or serum corticosterone levels (data not shown) in db/db mice. Therefore, the antiobesity effect of BRL35135A is independent of its effect on food intake and serum corticosterone levels. It has been hypothesized that, in rodents, the antiobese effects of β_3-adrenoceptor agonists are due to direct lipolysis of fat tissues and the increase in energy expenditure mediated by a proton-conductance pathway [16] that is under the control of β_3-adrenoceptors in brown adipose tissues [17]. It seems clear that these mechanisms play roles in mediating the antiobesity effects of BRL35135A in db/db mice.

In db/db mice, BRL35135A improved hyperglycemia at a low dose which did not affect body weight gain significantly, and increased the serum insulin levels at a dose of 0.05 mg/kg/day. However, when the doses of BRL35135A were increased, serum insulin levels decreased, as did serum glucose levels. These findings suggest that a dose of 0.05 mg/kg/day improved insulin resistance slightly in db/db mice, enabling them to respond to hyperglycemia. There-

TABLE 3. Effects of BRL35135A on glucose tolerance, serum insulin, and lipids levels and the side-effects in genetically obese Zucker fa/fa rats

	Lean fa/+ rats	fa/fa Rats			
			BRL35135A		
	Untreated	Vehicle	0.005 mg/kg	0.015 mg/kg	0.05 mg/kg
OGTT (mg · hr/dL)	425 ± 19	595 ± 49*	399 ± 21	316 ± 27†	315 ± 12†
(area under the curve for 0–120 min)	(71)	(100)	(67)	(53)	(53)
Insulin (μU/mL)	11 ± 1	374 ± 110*	150 ± 42‡	129 ± 32‡	141 ± 20‡
	(3)	(100)	(40)	(34)	(38)
Free fatty acid (μEq/L)	779 ± 62	1139 ± 139§	1123 ± 143	784 ± 45	652 ± 43†
	(68)	(100)	(99)	(69)	(57)
Triglyceride (mg/dL)	441 ± 20	2758 ± 318*	2126 ± 253	1291 ± 94‡	831 ± 103‡
	(16)	(100)	(77)	(47)	(30)
Total cholesterol (mg/dL)	32 ± 8	108 ± 9*	99 ± 9	63 ± 7‡	45 ± 4‡
	(30)	(100)	(92)	(58)	(42)
Heart rate (beats/min)		430 ± 8	436 ± 10	434 ± 9	449 ± 19
		(100)	(101)	(101)	(104)
Serum potassium (mEq/L)	3.48 ± 0.23	3.64 ± 0.12	3.35 ± 0.08	3.54 ± 0.07	3.79 ± 0.08
	(96)	(100)	(92)	(97)	(104)

BRL35135A was given p.o. daily for 6 weeks. The heart rate was measured 0.5 hr after the first oral administration. The serum potassium level was measured 0.5 hr after the last oral administration. In lean fa/+ rats, heart rate was not measured. The test to measure tolerance for oral glucose (2 g/kg) was performed 0.5 hr after the last drug administration. Each value is the mean ± SEM of six rats, and the percentages of values of vehicle-treated fa/fa rats are given in parentheses.

* $P < 0.01$, vs lean fa/+ rats (Student's t-test).

† $P < 0.05$, vs vehicle-treated rats (Bonferroni test).

‡ $P < 0.01$, vs vehicle-treated rats (Bonferroni test).

§ $P < 0.05$, vs lean fa/+ rats (Student's t-test).

fore, insulin secretagogue effects may be induced by high levels of glucose. It appears possible that when the dose of BRL35135A is increased, improvement in insulin resistance is sufficient to normalize serum glucose levels, but not sufficient to normalize serum insulin levels. These observations encouraged us to investigate the metabolic effects and the mechanism of action of BRL35135A in genetically obese Zucker fa/fa rats, an animal model with hyperinsulinemia and insulin resistance, at doses that did not affect their body weight significantly.

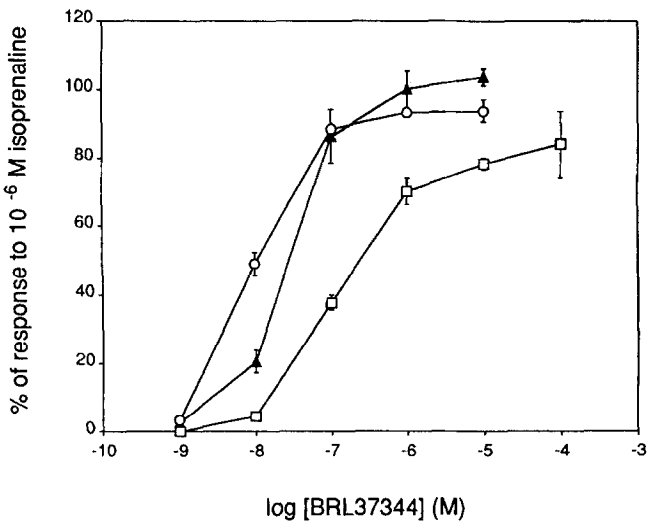

FIG. 3. Concentration–response curves for lipolysis induced by BRL37344 in rat mesenteric (▲), perirenal (○), and subcutaneous (□) adipocytes. Values are means ± SEM from three experiments.

Since BRL35135A tended to increase the food intake slightly in fa/fa rats, the body weight gain adjusted for energy intake was suppressed slightly at only 0.05 mg/kg/day. However, BRL35135A did not affect body weight gain significantly. It is worth noting that metabolic disorders including glucose intolerance, hyperinsulinemia, hypertriglyceridemia, and hypercholesterolemia were improved remarkably 0.005 and 0.015 mg/kg/day, doses at which BRL35135A had very little effect on body weight. Concerning fat distribution, slight reduction, especially of mesenteric fat, was observed at these doses. Therefore, the improvement of glucose intolerance and hyperlipidemia at low doses may be related to the selective reduction of mesenteric fat. Moreover, given that the most pronounced effect at the lowest dose was the improvement of hyperinsulinemia, it appears that BRL35135A not only reduces mesenteric fat but also improves insulin resistance via direct

TABLE 4. Tissue concentration of [^{14}C]BRL37344 in Wistar rats at 0.5 hr after oral administration of [^{14}C]BRL35135A

Tissue	Concentration (nM)
Plasma	687 ± 60
Brown fat	197 ± 20
Mesenteric white fat	656 ± 252
Perirenal white fat	138 ± 34
Subcutaneous white fat	73 ± 7

[^{14}C]BRL35135A was given p.o. at a dose of 1 mg/kg. Each value is the mean ± SEM for six animals.

effects on the target organs of insulin such as liver and muscle. Indeed, it has been reported that the major contribution of BRL35135A in improving insulin sensitivity in fa/fa rats is its suppression of endogenous glucose production [18]. In addition, the amelioration of hypertriglyceridemia by BRL35135A is due to its improvement of hyperinsulinemia via augmentation of insulin sensitivity. This appears to be the case, since the association of hyperlipidemia with insulin resistance is well recognized, and increase in hepatic very-low-density lipoprotein (VLDL)-triglyceride secretion during hyperinsulinemia and reduction in the rate of removal of VLDL-triglyceride from plasma by insulin resistance are thought to participate in the pathogenesis of hypertriglyceridemia [19, 20].

Although BRL37344 has primarily β_3-selective adrenoceptor agonist effects, it also has weak β_1- and β_2-activities [5]. In the present study of Zucker fa/fa rats, however, BRL35135A induced no tachycardia or hypokalemia, which are associated with β_1- and β_2-adrenoceptor activities, respectively [21, 22].

In conclusion, this study has shown that BRL35135A strongly improves glucose intolerance and dyslipidemia by selectively reducing visceral fat and by decreasing insulin resistance in peripheral organs. It can be expected, therefore, that BRL35135A will be quite useful clinically for the improvement of metabolic disorders arising from visceral fat obesity.

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