(−)-BM 13.0913: A New Oral Antidiabetic Agent That Improves Insulin Sensitivity in Animal Models of Type II (non–insulin-dependent) Diabetes Mellitus

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Insulin resistance is one of the key features of non–insulin-dependent diabetes mellitus (NIDDM). Therefore, a drug that causes an improvement in insulin sensitivity would be of great interest for the treatment of NIDDM. In addition to the insulin-sensitizing thiazolidinediones, we have found another class of insulin-sensitizing agents: the α-activated carbonic acids. (−)-BM 13.0913, a member of this class, was effective in improving insulin resistance in hyperinsulinemic and hypoinsulinemic insulin-resistant animal models of NIDDM. The 50% effective dose (ED_{50}) for the glucose-lowering action was 4, 2.4, and 8 mg/kg in ob/ob, yellow KK, and db/db mice, respectively. The ED_{50} for the insulin-lowering action was 14.5, 5, and 26 mg/kg. This rightward shift of the dose-response curve for insulin indicates that improving glucose homeostasis is the primary effect of the drug, followed by an insulin-decreasing action. This effect on glucose homeostasis may be brought about by sensitizing peripheral target tissues to the effects of insulin. An increase in deoxyglucose uptake and glucose oxidation measured in adipocytes from rats that had been treated for 14 days with (−)-BM 13.0913 supports this conclusion. Glucose uptake and oxidation was increased at all insulin concentrations tested, suggesting an improved responsiveness. Insulin sensitivity in adipocytes was not influenced by the drug. Studies in the moderately hypoinsulinemic, low-dose streptozotocin (STZ) diabetic rat with a residual insulin concentration showed a decrease in blood glucose concentrations, as well as a decrease in urinary glucose. Data obtained with STZ-diabetic rats with no residual insulin concentration and (−)-BM 13.0913 showed no effect on blood and urinary glucose, further supporting the insulin-sensitizing action of the drug. Additionally, no clinical signs of hypoglycemia were detected in any of the animal models tested. Furthermore, a decrease in serum nonesterified fatty acids (NEFA) and triglycerides was seen with an ED_{50} of 6 mg/kg. The (+)-enantiomer of BM 13.0913 showed no effect on glucose and insulin concentrations in any of the mice models tested. An enantioselective action was thus demonstrated. From these results, it is concluded that the (−)-enantiomer of BM 13.0913 may prove to be an active drug in treating insulin resistance as seen in NIDDM.

A NUMBER OF NEW oral antidiabetic agents have recently been found whose mode of action is different from that of sulfonylureas and biguanides, the drugs currently used for the treatment of non–insulin-dependent diabetes mellitus (NIDDM). All these agents, eg, ciglitazone,[1] CS-045,[2] pioglitazone,[3] and englitazone,[4] produced an improvement in insulin sensitivity in insulin-resistant rodent models, suggesting that there might be an amelioration in insulin resistance in man. Insulin resistance is thought to be a major contributing factor in the pathogenesis of NIDDM (for an overview, see DeFronzo et al[5]). All of the above-mentioned agents belong to the same chemical class of drugs, the thiazolidinediones. Here, we present studies with insulin-resistant animal models and the (−)- and (+)-enatiomers of BM 13.0913 [2-(4-methylphenoxy)-7-(4-chlorophenyl)-heptanic acid], members of a new class of insulin-sensitizing agents chemically unrelated to the thiazolidinediones. An enantioselective action will be demonstrated by comparing the results obtained with the two enantiomers.

MATERIALS AND METHODS

Materials

(+)- and (−)-BM 13.0913 (Fig 1) were synthesized in the chemical laboratories of Boehringer Mannheim (Mannheim, Germany) and were used in form of the Na salt. Porcine insulin was purchased from Novo Nordisk (Mainz, Germany), collagenase from Worthington Biochemical (Freehold, NJ), streptozotocin (STZ) from Boehringer Mannheim, ^{14}C-2-deoxyglucose from Sigma (Munich, Germany), and U-^{14}C-glucose from New England Nuclear (Dreieich, Germany).

Animals

Male C57BL/6J-ob/ob mice and their lean controls (either ob/+ or +/+) were obtained from Jackson Laboratories (Bar Harbor, ME) or from Bommice (Ry, Denmark). Female yellow KK mice were purchased from Clea (Kyoto, Japan), male db/db mice from Jackson Laboratories, and Lewis rats from Charles River Wiga (Sulzfeld, Germany). All animals were housed in standard animal cages, had free access to water, and were fed ad libitum with a normal laboratory chow. The ob/ob mice used for experiments were aged 12 to 14 weeks, yellow KK mice 18 weeks, and db/db mice 10 months. The Lewis rats were 8 to 10 weeks old.

Induction of Low-Dose STZ-Diabetes

Diabetes was induced by subcutaneous injection of a freshly prepared solution of STZ (65 mg/kg in saline solution). One week after diabetes induction, rats were treated with (−)-BM 13.0913 for 2 weeks. Blood glucose was determined before treatment and on days 3, 6, 9, 13, and 15 of drug treatment. Urinary volume and urinary glucose were determined on day 12 of substance administration.

Drug Administration and Blood Sampling

Drug dosages used in the experiments ranged from 3 to 100 mg/kg. (−)-BM 13.0913 and (+)-BM 13.0913 were prepared for oral administration as a suspension in 1% methyl cellulose. All doses are expressed as the amount of free acid administered daily. Blood samples for determining glucose concentration were collected by cutting the tip of the tail vein immediately before the first drug administration, as well as on the days shown in figure legends.

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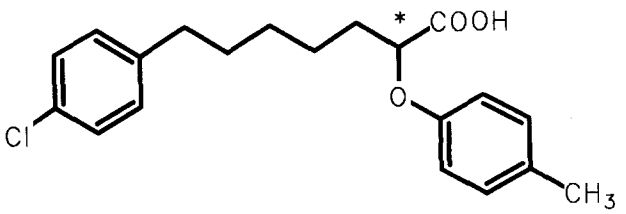

Fig 1. Chemical structure of BM 13.0913 (racemic BM 13.0913). *Chiral center.

Oral glucose tolerance tests were performed in conscious ob/ob mice to avoid the effect of anesthesia. For oral glucose tolerance tests, animals were given a 2-g/kg glucose solution; blood was obtained as described earlier at the indicated time points. At the end of the experimental period, animals were stunned and killed. The blood obtained was collected and centrifuged after clotting. The serum obtained was used for determination of insulin, triglycerides, nonesterified fatty acids (NEFA), and cholesterol. In addition, the liver was removed for determining triglycerides and cholesterol.

Analytical Methods

Blood glucose concentrations were determined with a Glucoquant test-combination (Boehringer Mannheim), on an Epos-Analyzer 5060 (Eppendorf-Gerätebau, Hamburg, Germany) according to the hexokinase method in hemolysate.[6] Serum insulin concentrations were determined using a commercially available Insulin-RIA-Kit 100 (Pharmacia Diagnostics, Uppsala, Sweden). Serum triglycerides were assayed after enzymatic cleavage of the triglycerides by a lipase, as described by Nägele et al.[7] Determination of serum cholesterol was based on an enzymatic method described by Siedel et al.[8] Triglycerides and cholesterol in liver were extracted with Folch-Sperry solution. Triglycerides were saponified with ethanolic KOH and determined photometrically according to methods reported by Eggstein and Kreutz[9] and Schmidt and von Dahl.[10] Cholesterol was assayed by the above-mentioned method.[8]

Preparation of Isolated Rat Adipocytes

Rats were administered different doses of (−)-BM 13.0913 for 14 days by gavage as described in figure legends. The corresponding control group was given only the vehicle (1% methyl cellulose) orally. At the end of the 14-day administration period, animals were killed and epididymal fat pads were removed and incubated at 37°C in collagenase-containing buffer (500 U/L, see below) under carbogen (95% O_2, 5% CO_2) in a shaking water bath at 80 rpm for 1 hour according to the method reported by Rodbell.[11] The suspension was filtered through a nylon sieve to remove undigested residue, and subsequently, the cell suspension obtained was centrifuged at 50 × *g*. The cells were washed three times with buffer containing 140 mmol/L NaCl, 10 mmol/L HEPES, 4.7 mmol/L KCl, 2.5 mmol/L KH_2PO_4, 1.25 mmol/L $MgSO_4$, 2.5 mmol/L $CaCL_2$, 4% fat-free bovine albumin, and 5 mmol/L glucose, pH 7.4, and once with buffer without glucose. For glucose oxidation studies, 5×10^5 cells were incubated in buffer with or without insulin. The reaction was started with U-^{14}C-glucose (7.4 kBq; final glucose concentration, 1 mmol/L). Incubation was stopped 1.5 hours later with H_2SO_4. $^{14}CO_2$ level was measured directly via an ionization chamber. For glucose uptake, 5×10^5 cells were incubated for 20 minutes at 37°C with or without insulin. The glucose uptake reaction was started by addition of ^{14}C-2-deoxyglucose (3.7 kBq; final glucose concentration, 0.5 mmol/L). After 3 minutes, ^{14}C-2-deoxyglucose uptake was stopped by addition of ice-cold phloretin solution (1 mmol/L). This mixture was immediately coated with silicone oil and centrifuged. The adipocytes were removed, and radioactivity was counted in a liquid scintillation counter.

Statistical Analysis

The data are expressed as the mean ± SEM. Statistical significance of differences between experimental groups was calculated by the Mann-Whitney test for unpaired samples, α-adjusted by the Bonferroni method. A value of P less than .05 was considered significant. The area under the curve (AUC) was determined by the trapezoidal rule; 50% effective dose (ED_{50}) values were calculated graphically.

RESULTS

Influence of (−)-BM 13.0913 on Blood Glucose and Insulin Concentrations in ob/ob, db/db, and Yellow KK Mice

Studies with ob/ob mice were performed over 5 days. As presented in Fig 2, a significant decrease in fed blood glucose was already obtained at a dose of 5 mg/kg/d (−)-BM 13.0913; the ED_{50} was 4 mg/kg/d, and the maximum decrease was achieved with approximately 25 to 30 mg/kg/d. Figure 2 also shows a dose-dependent decrease in fed insulin concentrations compared with the dose-response curve for the blood glucose–lowering effect. It is evident that there is a shift to the right. The ED_{50} was 14.5 mg/kg/d, and the maximally active dose was approximately 75 mg/kg/d.

Experiments with db/db (Fig 3) and yellow KK (Fig 4) mice also showed a dose-dependent decrease in fed blood glucose and fed insulin concentrations, thus confirming the results obtained with ob/ob mice. ED_{50} values for decreases in blood glucose and insulin concentrations measured in db/db mice after 15 days of dosing were 8 and 26 mg/kg/d, respectively. On day 5 and day 9, results obtained with yellow KK mice showed that a dose of 5 mg/kg/d does not achieve maximal effects. At day 12, it is obvious that this dose is also nearly maximally active. As for ob/ob and db/db mice, there was also a shift to the right as compared with the dose-response curves for insulin (ED_{50}, ~5 mg/kg/d) and glucose (ED_{50}, ~2.4 mg/kg/d). ED_{50} values for

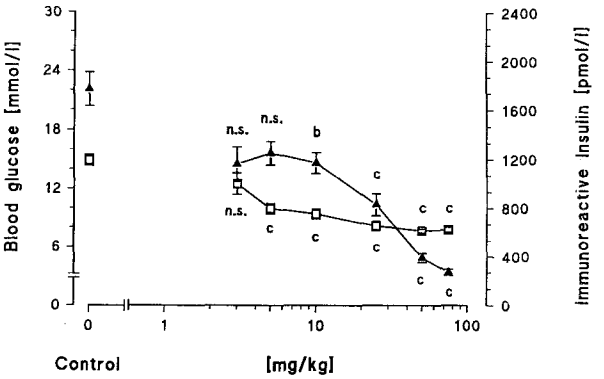

Fig 2. Plasma glucose (□) and insulin (▲) levels in ob/ob mice measured 2 hours after the fifth daily oral dose of vehicle or 3 to 75 mg · kg^{-1} · d^{-1} (−)-BM 13.0913.Na. Each value represents the mean ± SEM for 10 to 40 animals. $^bP < .01$, $^cP < .001$: vehicle *v* drug-treated animals.

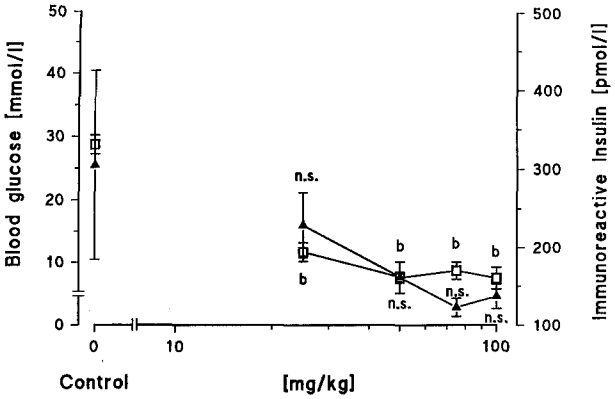

Fig 3. Plasma glucose (□) and insulin (▲) concentrations in db/db mice measured 2 hours after the 15th daily oral dose of vehicle or 25 to 100 mg · kg^{-1} · d^{-1} (−)-BM 13.0913.Na. Each value represents the mean ± SEM for 10 animals. n.s., nonsignificant; $^{b}P < .01$, vehicle *v* drug-treated animals.

yellow KK mice were obtained on day 15 of substance administration. No glucose-lowering action exceeding normoglycemia was detected in ob/ob, yellow KK, and db/db mice even at a dose of 100 mg/kg/d (data not shown).

STZ-Diabetic Rats With a Residual Insulin Concentration

Chronic responses of blood glucose, insulin, and urinary glucose to (−)-BM 13.0913 were also studied in the STZ-diabetic rat with a residual insulin concentration, another model of insulin resistance.[12] Blood glucose was evaluated over 14 days to investigate whether tachyphylactic effects occur. Blood glucose was also decreased in this model of insulin resistance, and there was no evidence of tachyphylaxia (Fig 5). Figure 6 demonstrates that the decreased blood glucose concentration was also reflected by a decreased urinary glucose concentration (urinary volume and urinary glucose determinations were performed on day 12 of substance application with the aid of metabolic cages).

Oral Glucose Tolerance Test in (−)-BM 13.0913–Treated ob/ob Mice

The postprandial hyperglycemia seen in untreated ob/ob mice was dose-dependently improved by (−)-BM 13.0913 (Fig 7). The AUC (area under glucose tolerance curve) at a dose of 50 mg/kg/d was comparable to a nondiabetic control value (Table 1).

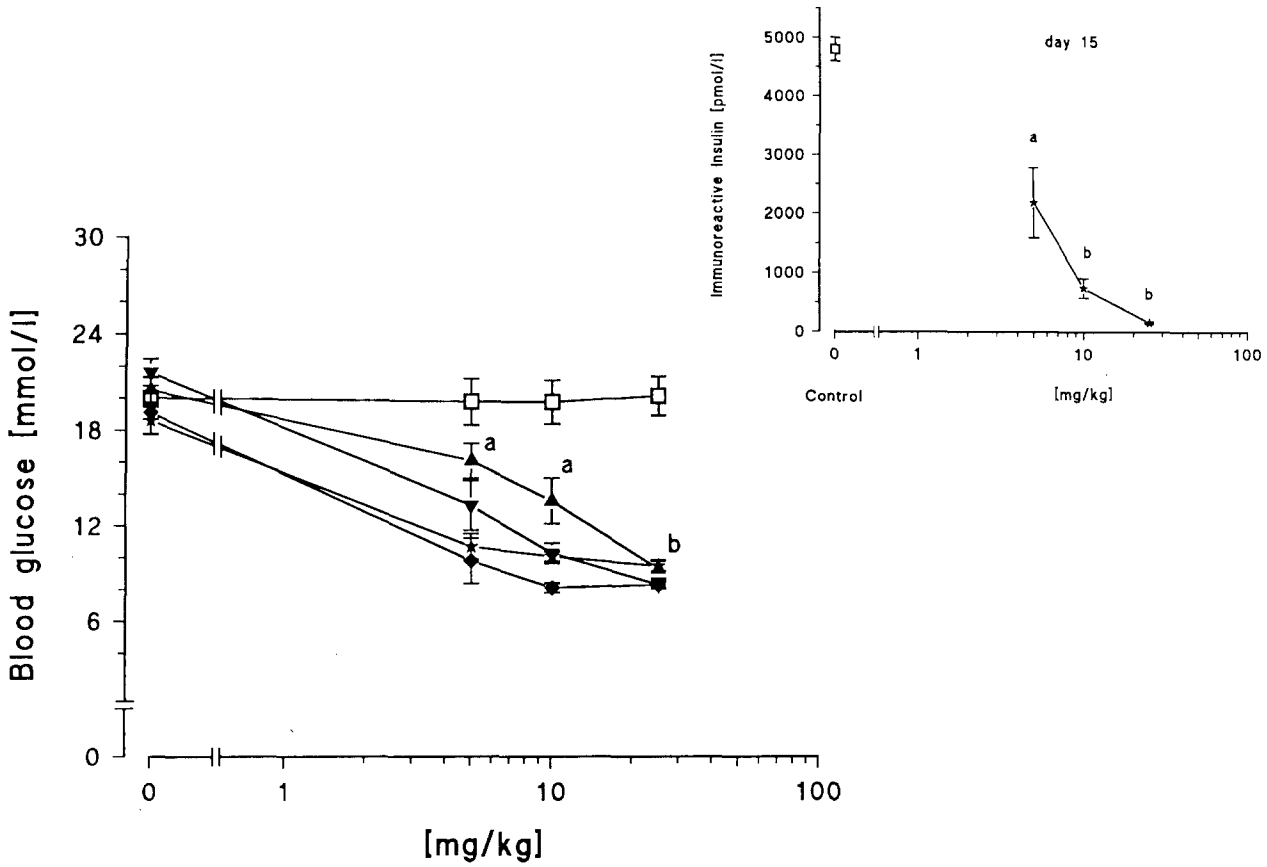

Fig 4. Plasma glucose and insulin concentrations in yellow KK mice measured 2 hours after 4 different application periods (□, pretreatment; ▲, 5 days; ▼, 9 days; ◆, 12 days; ★, 15 days) in the same animals with 5 to 25 mg/kg (−)-BM 13.0913.Na or vehicle. Values represent the mean ± SEM for 8 mice. $^{a}P < .05$, ^{b}P M .01: vehicle *v* drug-treated animals. Significance in this figure are shown only for the 5-day administration period for clarity. Significance values for administration periods of > 5 days are not > .01.

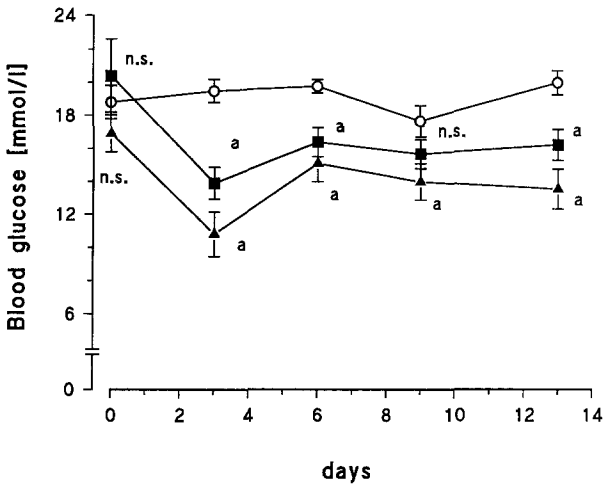

Fig 5. Blood glucose levels in low-dose STZ-diabetic rats measured after different periods of administration of (−)-BM 13.0913.Na (■, 25 mg/kg; ▲, 50 mg/kg) or vehicle (○). Values represent the mean ± SEM for 10 rats. n.s., nonsignificant; $^{a}P < .05$, vehicle *v* drug-treated animals at corresponding day of administration.

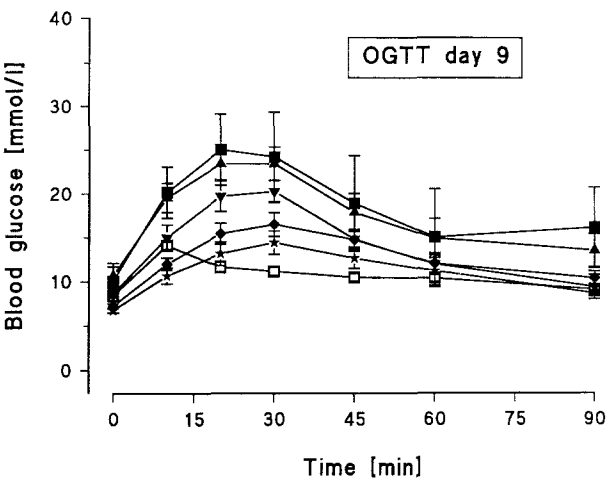

Fig 7. Effect of (−)-BM 13.0913.Na on glucose tolerance in ob/ob mice (2 g glucose/kg body weight). (−)-BM 13.0913.Na was orally administered for 9 days: □, nondiabetic control; ■, vehicle-treated control; ▲, 5 mg/kg; ▼, 10 mg/kg; ◆, 30 mg/kg; ★, 50 mg/kg. Values represent the mean ± SEM for 10 rats. Corresponding AUCs are depicted in Table 1.

Effect of (+)-BM 13.0913 on Blood Glucose and Insulin in ob/ob, db/db, and Yellow KK Mice

In contrast to the results obtained with (−)-BM 13.0913, (+)-BM 13.0913 was not effective in decreasing blood glucose concentrations and insulin concentrations in any of the insulin-resistant mice models tested, even at those doses that were maximally effective for the (−)-enantiomer. Data obtained with ob/ob mice are shown as an example (Fig 8). Thus, an enantioselective action can clearly be demonstrated.

Effect of (−)-BM 13.0913 on Serum Lipids

In addition to the action of decreasing glucose and insulin levels, there was a clear effect on serum NEFA and triglyceride concentrations in all mice models tested. As shown for blood glucose and insulin, these effects were dose-dependent. For example, ED_{50} values for the decreasing action on NEFA and triglyceride concentrations in ob/ob mice were 6 mg/kg/d for both parameters. No change was found in serum cholesterol levels.

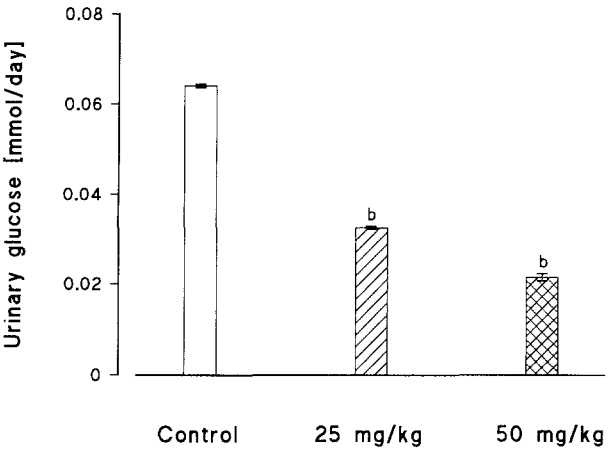

Fig 6. Urinary glucose in low-dose STZ-diabetic rats after 12 days of (−)-BM 13.0913.Na: (▨, 25 mg/kg, (▩) 50 mg/kg. Values represent the mean ± SEM for 10 rats. $^{b}P < .01$, vehicle *v* drug-treated rats.

Glucose Uptake and Glucose Oxidation Studies With Rat Adipocytes

The results presented so far led us to speculate that (−)-BM 13.0913 improves insulin sensitivity in peripheral tissues. To test this hypothesis, we performed experiments with fat cells from rats treated with the drug for 14 days. Figure 9 presents the effect of drug treatment on 2-deoxyglucose uptake at various insulin concentrations in comparison to values for vehicle-treated controls. Insulin caused a concentration-dependent stimulation of 2-deoxyglucose uptake in both control and drug-treated adipocytes. (−)-BM 13.0913 in feed significantly increased the rate of glucose uptake at all insulin concentrations without changing the

Table 1. Changes in AUC for Glucose Tolerance After 2 g/kg Oral Glucose in ob/ob Mice Treated With (−)-BM 13.0913.Na

	AUC (mmol · L^{-1} · 90 min)	
	ob/ob Mouse	Nondiabetic ob/ob Mouse Control
Vehicle	765 ± 63	288 ± 47
(−)-BM 13.0913.Na (mg/kg/d)		
5	638 ± 51*	—
10	537 ± 45†	—
30	516 ± 54†	—
50	386 ± 50‡§	—

NOTE. Values are the mean ± SEM; n = 10.
*NS *v* vehicle-treated ob/ob mouse.
†$P < .01$ *v* vehicle-treated ob/ob mouse.
‡$P < .001$ *v* vehicle-treated ob/ob mouse.
§NS *v* vehicle-treated nondiabetic ob/ob mouse control.

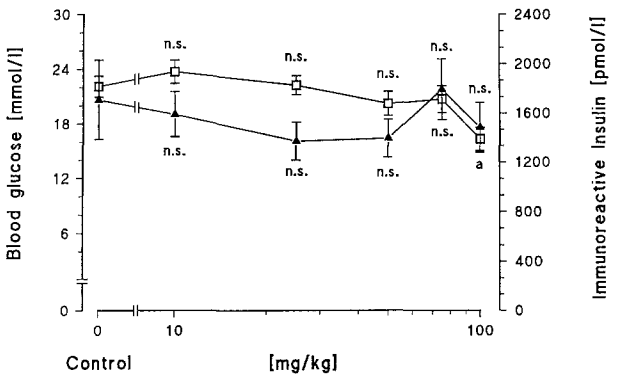

Fig 8. Effect of (+)-BM 13.0913.Na on blood glucose (□) and insulin (▲) concentrations in ob/ob mice. Values represent the mean ± SEM for 10 animals. n.s., nonsignificant; $^{a}P > .05$, vehicle (control) *v* drug-treated mice.

50% effective concentration (EC_{50}) value (0.1 nmol/L). Basal glucose uptake also was not affected by the drug.

The influence of the drug on glucose oxidation is depicted in Fig 10. As in the glucose uptake studies, rats were dosed for 14 days. The glucose oxidation rate was enhanced at all doses and all insulin concentrations tested as compared with values for nontreated controls. This enhancement was most prominent at those insulin concentrations that were responsible for maximal insulin stimulation. As seen in the glucose uptake studies, no changes were detected in EC_{50} concentrations (0.25 nmol/L).

DISCUSSION

Insulin resistance is a prominent feature of NIDDM.[5] Animal models of NIDDM such as ob/ob,[13] yellow KK,[14,15] and db/db[13] mice and STZ-diabetic rats with residual

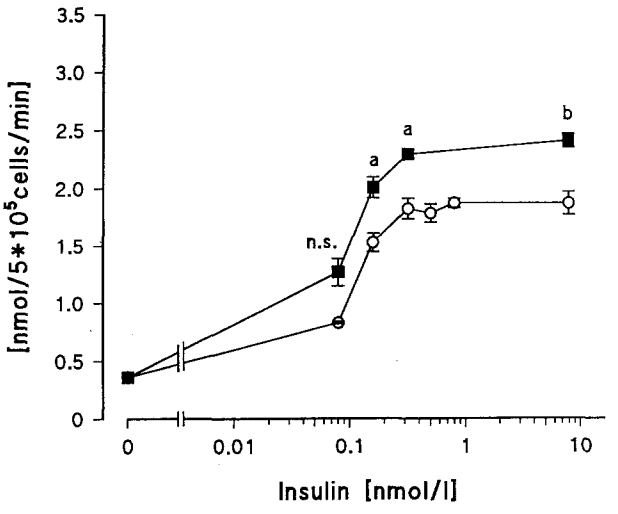

Fig 9. Ability of adipocytes in Lewis rats to promote ^{14}C-deoxyglucose uptake at various insulin concentrations. (−)-BM 13.0913.Na 5 mg/kg (■) was administered via gavage for 14 days. (○) Control values (vehicle). Values are the mean ± SEM from 6 determinations. n.s., nonsignificant; $^{a}P < .05$, $^{b}P < .01$: vehicle-treated *v* drug-treated adipocytes.

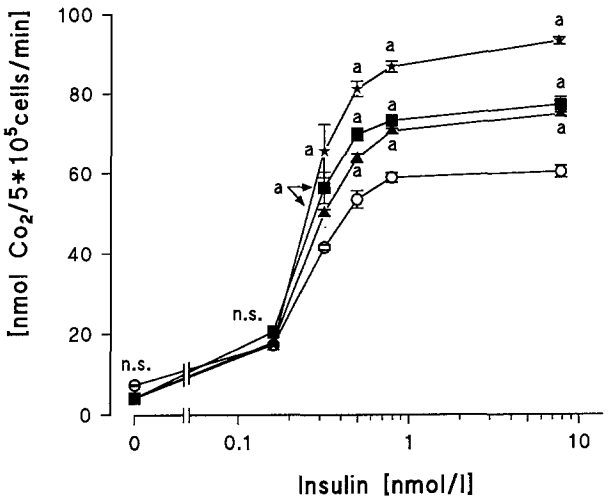

Fig 10. Ability of adipocytes in Lewis rats to induce U-^{14}C-glucose oxidation at various insulin concentrations: 5 mg/kg (■), 10 mg/kg (▲), and 25 mg/kg (★). (−)-BM 13.0913.Na was administered via gavage for 14 days. (○) Control values (vehicle). Values are the mean ± SEM from 6 determinations. n.s., nonsignificant; $^{a}P < .05$, vehicle-treated *v* drug-treated adipocytes.

insulin concentration[12] resemble various abnormalities of insulin resistance also seen in NIDDM patients. Although the genetic models ob/ob and yellow KK mice show some overall similarities such as hyperglycemia, hyperinsulinemia, obesity, and an early phase of insulin resistance, some differences exist. The db/db mouse also represents a genetic form of insulin resistance, but later on these animals develop severe hyperglycemia associated with islet cell degeneration even when insulin levels are reduced to a near-normal range. Thus, this progression of the diabetic syndrome appears to be similar to that in obese human NIDDM subjects.[16] By testing the new substance (−)-BM 13.0913 with these three mice models, it should be possible to observe effects of this new drug on different phases of insulin resistance, as well as in different genetic constellations, all of which possibly resemble that of NIDDM. The STZ-diabetic rat with moderate insulin deficiency was used[12] to test if an action of the drug could also be demonstrated in a hypoinsulinemic insulin-resistant model.

In comparing the ED_{50} values for glucose and insulin, the ED_{50} for the insulin-lowering action is shifted to the right. The same has also been shown for pioglitazone in yellow KK mice.[3] This means that the insulin concentrations are only influenced if the blood-glucose concentration is decreased to a certain level. This reduction in blood glucose, followed by a dose-related decline in insulin concentrations, indicates that the insulin resistance present in these three mice models is positively influenced by (−)-BM 13.0913. These data are supported by the fact that pathologic postprandial glucose fluctuations measured in ob/ob mice (Fig 7) were reduced dose-dependently to near-normal levels comparable to those in a nondiabetic ob/ob mouse control (Table 1).

Another model of insulin resistance is the STZ-diabetic

rat with moderate insulin deficiency. In contrast to the above-mentioned hyperinsulinemic insulin-resistant mice models, this is a hypoinsulinemic model. However, compared with rats made diabetic with a high intravenous dose of STZ dissolved in citrate buffer, rats treated subcutaneously with STZ dissolved in NaCl retain residual insulin concentrations. This model therefore represents a more physiologic state of insulin resistance not influenced by a catabolic state as seen with a high dose of STZ.[12] Data ascertained in our laboratory gave an immunoreactive insulin level of 18 to 25 μU/mL in non–STZ-treated rats, 10 to 12 in rats treated with STZ dissolved in NaCl, and 3 to 5 in rats treated with STZ dissolved in citrate buffer. These data are in good accordance with data published by Kobayashi and Olefsky,[12] for example. As shown in Figs 5 and 6, (−)-BM 13.0913 decreased blood glucose concentrations and urinary glucose. Data obtained with 100 mg/kg/d (−)-BM 13.0913 and diabetic rats treated with STZ dissolved in citrate buffer (data not shown) showed no evidence of a glucose-lowering action. From these results, it is concluded that (−)-BM 13.0913 can only act if the drug and insulin are both present. This means that (−)-BM 13.0913 sensitizes tissue to the effects of insulin with respect to the glucose-lowering action. In an accompanying report,[17] this sensitizing action is shown directly in the insulin-resistant fa/fa rat by means of the euglycemic-hyperinsulinemic glucose clamp technique.

How is this sensitizing action brought about? As shown in Figs 9 and 10, (−)-BM 13.0913 was effective in increasing glucose uptake and glucose oxidation in rat fat cells. Thus, the rat fat cell, which is a model of a peripheral target tissue of insulin action, shows that there is an improvement in peripheral insulin action. According to the considerations of Kahn,[18] a postreceptor defect in insulin action leads to a proportionate reduction in biological effects at all insulin concentrations. However, a receptor defect leads to a rightward shift in the insulin concentration-response curve. The results presented here demonstrate an enhanced glucose uptake and oxidation rate at all insulin concentrations. Based on these considerations, one can conclude that the improvement in insulin action in this model takes place at the postreceptor level. It remains to be elucidated which target is influenced by the drug.

In contrast to (−)-BM 13.0913, studies with (+)-BM 13.0913 showed no reduction in glucose or insulin in any of the mice models tested. With reference to this stereoselective action, one can speculate that the mode of action is a highly specific one. Furthermore, it is well known that receptors discriminate between enantiomers in a stereoselective manner.[19] If there is a high degree of concordance concerning the biological effect and drug-receptor interaction, such a tool offers the possibility of searching for the target(s) implicated in the pathogenesis of insulin resistance in animal models of NIDDM and human NIDDM.

In addition to the reduction in glucose and insulin, (−)-BM 13.0913 also decreased triglyceride and NEFA concentrations. This effect has also been shown for the thiazolidinediones.[2-4] The decrease in triglyceride and NEFA concentrations in serum was preceded by a reduction in glucose concentrations (data not shown). Therefore, the glucose-lowering action of the drug is not the consequence of an improved lipid metabolism, but rather the consequence of a direct interaction with glucose homeostasis. Nevertheless, an indirect improvement in glucose utilization via the glucose–fatty acid cycle[20] could contribute to the overall sensitizing effect of (−)-BM 13.0913.

In summary, (−)-BM 13.0913 is effective in decreasing glucose and insulin in hyperinsulinemic, insulin-resistant animal models, eg, ob/ob, yellow KK, and db/db mice. No such action was obtained with the (+)-enantiomer. A decrease in blood glucose was also detected in partially hypoinsulinemic STZ-diabetic rats. Even with high doses of the drug, clinical hypoglycemia was not seen. (−)-BM 13.0913 increased glucose uptake and glucose oxidation in rat fat cells, suggesting a peripheral site of action. In addition to the improvement in glucose homeostasis, an amelioration in the lipid status was shown. From these results, it is concluded that (−)-BM 13.0913 might be an effective drug for treating insulin resistance as seen in NIDDM.

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