Improved Glycemic Control in C57Bl/KsJ (*db/db*) Mice After Treatment with the Thermogenic β-Adrenoceptor Agonist, BRL 26830

M. J. CARROLL, CAROLYN A. LISTER, M. V. SENNITT, N. STEWART-LONG, AND M. A. CAWTHORNE

SUMMARY

BRL 26830, (R*,R*)-(±)-methyl-4-(2-[{2-hydroxy-2-phenylethyl}amino]propyl)-benzoate, is a new type of β-adrenoceptor receptor agonist that combines antihyperglycemic and thermogenic properties. In C57Bl/KsJ *db/db* mice, treatment with BRL 26830 (50 mg of the hemifumarate salt/kg diet) decreased blood glucose concentration and normalized water intake. As judged by the normalization of polydipsia, BRL 26830 was effective within 2 days and the effect was maintained throughout a treatment period of up to 11 wk. Treatment of *db/db* mice with BRL 26830 resulted in an increase in both plasma and pancreatic insulin concentrations and a partial restoration of first-phase insulin secretion by the isolated, perfused pancreas in response to a high (16.7 mM) glucose pulse. Given acutely, BRL 26830 increased energy expenditure in both fed and fasted *db/db* mice. When given chronically, BRL 26830 increased significantly the dietary and thermoregulatory component of metabolic rate. It is suggested that the antidiabetic and thermogenic properties of BRL 26830 are linked and that blood glucose acts either directly or indirectly as a substrate for thermogenesis. DIABETES 1985; 34:1198–1204.

The C57Bl/KsJ *db/db* mouse[1] is a useful animal model for the study of the metabolic and pathologic consequences[2] of type II (non-insulin-dependent) diabetes. In these mice, hyperinsulinemia can be demonstrated at 10 days of age[3] and persists until 4–5 mo, when blood insulin levels fall in association with pancreatic islet β-cell atrophy.[4] At approximately 1 mo of age, the *db/db* mice show increased fat deposition and progressive weight gain and obesity, which peak at 4 mo. Blood glucose concentrations are modestly elevated at 4 wk of age, but by 2 mo the mice have profound hyperglycemia and glycosuria.[5] By 4–5 mo of age, the mice exhibit aspects of diabetic nephropathy.[6]

It has been demonstrated that careful restriction of the dietary intake[7] and the use of high fiber diets[8] can reestablish some degree of control of blood glucose in *db/db* mice. Of the pharmacologic agents available, only the fatty acid oxidation inhibitor, McN-3802,[9] the combined amylase-disaccharidase inhibitor acarbose,[10] and ciglitazone[11] have been shown conclusively to be effective in lowering blood glucose.

BRL 26830, (R*,R*)-(±)-methyl-4-(2-[{2-hydroxy-2-phenylethyl}amino]propyl)-benzoate (Figure 1), is an example of a novel β-adrenoceptor agonist with thermogenic and antihyperglycemic activity.[12,13] It is chemically unrelated to either the sulfonylureas or biguanides.

In previous studies, BRL 26830 has been found to improve glucose tolerance in acute studies in normal rats and mice, but was without effect in streptozocin- or alloxan-diabetic rodents.[13] In the present studies, the effect of chronic treatment with BRL 26830 on blood glucose, plasma and pancreatic insulin concentrations, and glucose-stimulated insulin secretion in C57Bl/KsJ *db/db* mice has been examined.

MATERIALS AND METHODS

Animals. Female, diabetic (C57Bl/KsJ db^+/db^+) and lean heterozygotes (C57Bl/KsJ $db^+/{}^+m$) mice were obtained from the Jackson Laboratory, Bar Harbour, Maine.

The mice were housed 5/cage and maintained in a light-controlled (lights on 0600–1800 h) and temperature-controlled (23 ± 1°C) environment. The mice were allowed free access to food (Oxoid rat and mouse breeders diet in powder form; H. C. Styles, Bewdley, Worcs., United Kingdom) and water throughout the study. BRL 26830 (as the hemifumarate salt) and glibenclamide (a gift from Roussel, London, United Kingdom) were incorporated into the diet.

General experimental design. In the first experiment, *db/db* mice and lean littermates that were 5–6 wk old at the start of the experiment were studied for a 60-day period. Ten lean and 15 *db/db* mice were given the control diet and a further 15 *db/db* mice were given the same diet, supplemented with BRL 26830 (50 mg/kg diet). The mice were weighed on days

From the Beecham Pharmaceuticals Research Division, Great Burgh, Epsom, Surrey, United Kingdom.
Address reprint requests to Dr. M. A. Cawthorne at the above address.
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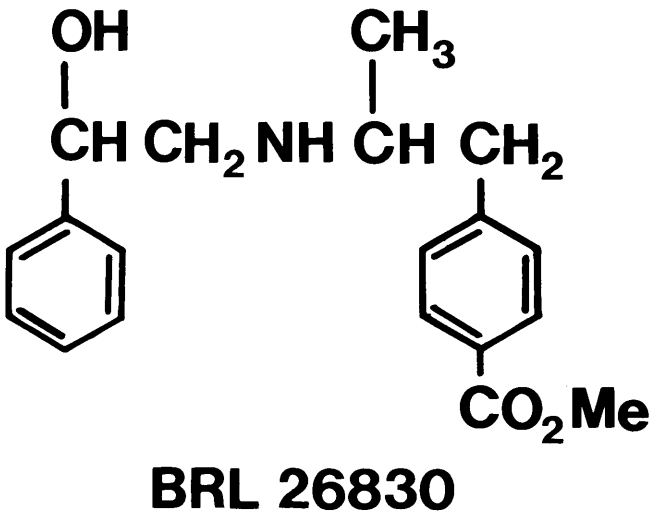

FIGURE 1. BRL 26830, (R*,R*)-(±)-methyl-4-(2-[{2-hydroxy-2-phenylethyl}amino]propyl)-benzoate, an example of a novel β-adrenoceptor agonist with thermogenic and antihyperglycemic activity.

1 and 14 and then at 7-day intervals thereafter. Water intake was measured daily. Blood for glucose determination was obtained at mid-day on day 1 and then at approximately 2-wk intervals. After 44 days of treatment, blood glucose concentration was measured at 2-h intervals throughout a continuous 24-h period. Hemoglobin A_1 (HbA_1) was measured after 37 days of treatment. The experiment was terminated after 70 days, when blood was obtained for the determination of plasma immunoreactive insulin concentration. The pancreatic insulin content was also measured and the composition of the residual carcass determined.

In the second experiment, the mice were 4–5 wk old at the start of the experiment, which lasted 11 wk. Ten lean and 14 *db/db* mice were given the control diet. Groups of 10 mice were given the same diet supplemented, respectively, with glibenclamide (50 mg/kg diet) or BRL 26830 (50 mg/kg diet). In addition to the measurements made in the first experiment, food consumption was determined daily.

Glucose, HbA_1, and insulin measurements. Blood for glucose estimation was obtained from the cut tip of the tail. Ten microliters of blood was hemolysed with 1 ml 1.0 mM maleimide containing 0.04 mM digitonin. The glucose content of the hemolysate was determined using the hexokinase method on a Clinicon Corona autoanalyzer.[14]

HbA_1 was measured in hemolysed blood by a modification of the ion-exchange column method of Schnek and Schroeder[15] using a Boehringer reagents kit (B.C.L., Lewes, E. Sussex, United Kingdom). The results are expressed as a percentage of total hemoglobin.

At the end of the experiment, the mice were killed by cervical dislocation and blood was taken from severed neck arteries into heparinized tubes. The plasma immunoreactive insulin concentration was measured by a double-antibody radioimmunoassay[16] using insulin-binding reagent (Wellcome Labs, Beckenham, United Kingdom). Human insulin was used as standard. Pancreatic tissue was extracted in acid-ethanol[17] and the insulin content determined by the same radioimmunoassay procedure.

Measurement of energy expenditure. Energy expenditure and thermogenic responses to an acute dose of BRL 26830 were determined by indirect calorimetry.[12,18] Briefly, mice were housed in plastic cages with slatted steel tops. Each cage was housed inside a wooden box through which air was drawn. In the experimental system, 15 boxes are arranged in parallel. By the use of a valve system, the expired, dried air from each box is sampled at 15-min intervals and the oxygen content determined by paramagnetic resonance (Taylor Servomax, Crowborough, Sussex, United Kingdom). Weir[19] has shown that energy expenditure can be calculated from the flow of dry air leaving each box of mice and the fall in percentage oxygen content of the air as it passes through each box. Oxygen consumption cannot be calculated from these measurements, since this requires that the flow of dry air into, as well as out of, the box is known. These two values are equal only when the respiratory quotient is one. If a respiratory quotient of one is assumed (carbohydrate combustion) when in fact it is 0.72 (fat combustion), oxygen consumption is underestimated by 6.3%. However, the energy produced per liter of oxygen consumed is 6.3% less for carbohydrate than for fat combustion. These factors are so similar that energy expenditure can be estimated very accurately without à knowledge of respiratory quotient.

TABLE 1
Effect of BRL 26830 and glibenclamide on the mid-day blood glucose concentration in *db/db* mice

Days of treatment	Control *db/+*	Control *db/db*	BRL 26830-treated *db/db*	Glibenclamide-treated *db/db*
Experiment 1				
1	6.5 ± 0.2 (10)	20.5 ± 2.2 (15)	22.1 ± 2.3 (15)	—
16	6.5 ± 0.2 (10)	25.3 ± 1.5 (15)	6.9 ± 0.4 (15)*	—
29	6.2 ± 0.2 (10)	30.6 ± 3.8 (15)	6.0 ± 0.2 (15)*	—
44	6.6 ± 0.4 (10)	26.0 ± 1.7 (15)	7.5 ± 0.9 (15)*	—
60	6.5 ± 0.3 (10)	24.5 ± 1.6 (15)	10.1 ± 1.4 (15)*†	—
Experiment 2				
1	7.0 ± 0.2 (9)	17.8 ± 1.6 (14)	17.3 ± 2.3 (10)‡	18.9 ± 2.4 (10)§
14	7.0 ± 0.5 (9)	21.5 ± 0.9 (14)	7.1 ± 0.6 (10)*	21.1 ± 1.6 (10)§
39	4.9 ± 0.1 (9)	20.0 ± 0.5 (14)	7.4 ± 0.9 (9)*†	19.3 ± 0.8 (10)§
54	4.6 ± 0.3 (9)	22.7 ± 0.9 (14)	10.8 ± 1.8 (9)*‡	26.2 ± 1.3 (10)§
76	6.1 ± 0.2 (9)	22.4 ± 0.8 (14)	12.0 ± 2.3 (9)*†	21.9 ± 2.0 (10)§

The number of mice in each group is given in parentheses; the values are expressed as mean ± SEM.
The degree of significance of treatment effect is calculated versus *db/db* controls: *$P < 0.001$; and versus *db/+* controls: †$P < 0.05$, ‡$P < 0.01$, and §$P < 0.001$.

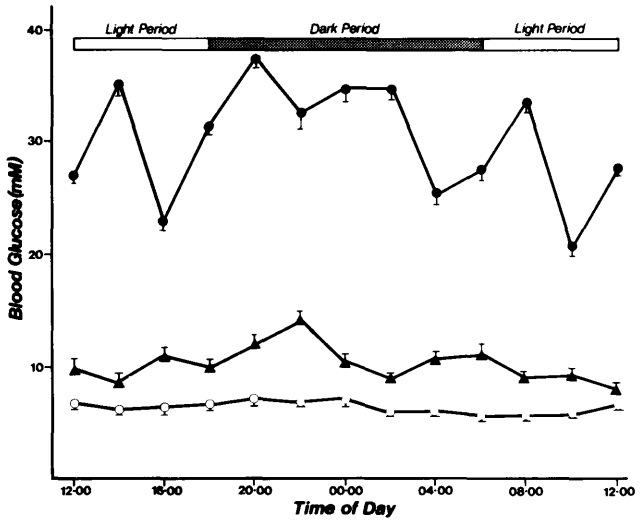

FIGURE 2. Diurnal pattern of blood glucose concentration of control *db*/+ (○) and *db*/*db* (●) mice, and *db*/*db* mice treated with BRL 26830 (▲). Results are the mean ± SEM of five values at each time point. The difference between BRL 26830-treated and control *db*/*db* mice was significant at all time points ($P < 0.001$).

Experiments were carried out either on fed mice at the normal animal house temperature of 23°C (total energy expenditure) or in fasted mice at thermoneutrality (29°C). In the latter case, in which basal metabolic rate is determined, food was removed and the mice were placed in a thermoneutral environment for 2 h before the measurement of energy expenditure.

Pancreas perfusion. Separate groups of animals were used for these experiments, but they were treated in an identical manner to those described above. At the time of carrying out the perfusion, the mice were 10–13 wk old and had received BRL 26830 for 3–5 wk.

The pancreas was perfused according to the method of Dunmore and Beloff-Chain[20] with minor modifications. A catheter was placed in the abdominal aorta and the pancreas was perfused through the celiac artery and superior mesenteric artery at the rate of 1.0 ml/min. The pancreas was not removed from the mouse, but the pancreatic circulation was isolated. In each experiment, the pancreas was preperfused for 10 min with 5.6 mM glucose. The pancreas was then perfused for a further 10-min period with 5.6 mM glucose followed by a 10-min perfusion with 16.7 mM glucose. The perfusate was collected at 1-min intervals for the assay of insulin.[16]

Statistics. All data are reported as means ± SEM (number of samples) except for those results given in Figure 3, which are mean ± SD. The differences were evaluated by the Student *t*-test.

RESULTS

Blood glucose. The mid-day blood glucose concentration of control *db*/*db* mice was four to five times higher than in the lean mice. In both experiments, treatment of the *db*/*db* mice with BRL 26830 (50 mg/kg diet) produced a highly significant reduction in the blood glucose concentration throughout the experimental period (Table 1). Indeed, for much of the experiment, the blood glucose concentrations in the BRL 26830-treated mice were not significantly different from those of the lean mice. This contrasted with the glibenclamide treatment, which had no significant effect on the mid-day blood glucose concentration (Table 1).

The blood glucose concentration of *db*/*db* mice is dependent on the feeding pattern of the mice and, therefore, samples taken at mid-day may not be representative of the total 24-h cycle. Thus, to ensure the validity of the mid-day measurement, the blood glucose concentration was determined at 2-h intervals throughout a continuous 24-h period. However, to minimize any interference with the normal behavior of the mice, only five mice in each treatment group were bled at each time point. Blood samples from individual *db*/*db* mice were obtained, therefore, at 6-h intervals. As shown in Figure 2, the blood glucose concentration of the untreated *db*/*db* mice was >20 mM throughout the 24-h period, and peaked at >35 mM. Treatment of *db*/*db* mice with BRL 26830 produced a significant reduction in the blood glucose concentration at all the time points.

HbA_1. In both experiments, BRL 26830A produced a significant reduction in the HbA_1 content, and after 37–46 days of

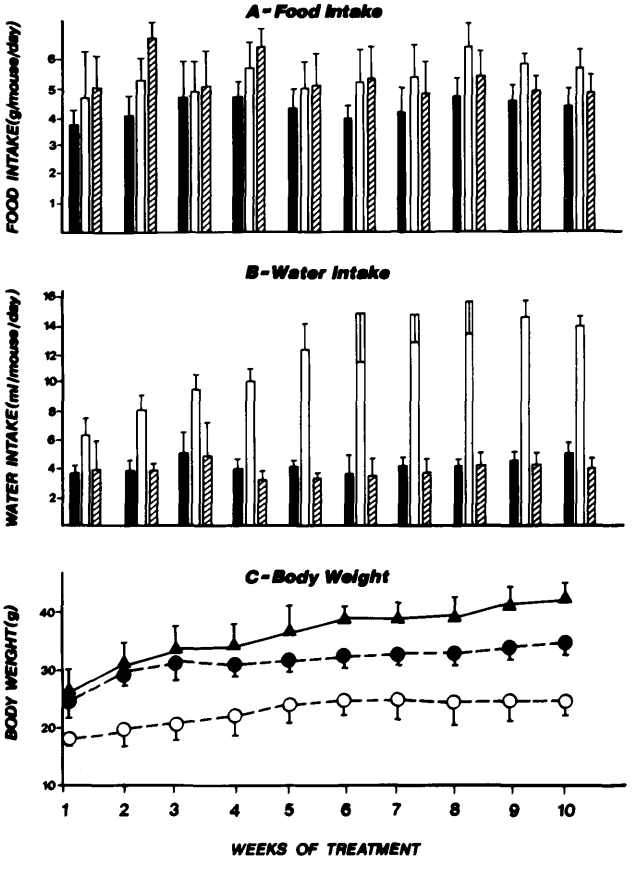

FIGURE 3. Food intake, water intake, and body weight in C57Bl/KsJ *db*/+ and *db*/*db* mice; untreated *db*/+ (○---○, solid bar), untreated *db*/*db* (●---●, open bar), and *db*/*db* mice treated with BRL 26830 (▲—▲, hatched bar). Results are given as mean ± SD. BRL 26830 was admixed with food at 50 mg/kg concentration. Food intake of the BRL 26830-treated mice was increased significantly ($P < 0.001$) during week 2 and reduced significantly ($P < 0.05$) during weeks 8, 9, and 10 relative to that in untreated *db*/*db* mice. The water intake of the untreated *db*/*db* mice was increased significantly ($P < 0.001$) relative to that in both *db*/+ mice and BRL 26830-treated *db*/*db* mice. The body weight of the BRL 26830-treated *db*/*db* mice was increased significantly ($P < 0.05$) from week 4 onward.

TABLE 2
Effect of BRL 26830 and glibenclamide on HbA_1 content, plasma insulin, and pancreatic insulin in *db/db* mice

Genotype and treatment	HbA_1	Plasma insulin (μU/ml)	Pancreatic insulin (U/g)
Experiment 1			
db/+	3.2 ± 0.2 (10)‡	76 ± 22 (5)	5.2 ± 0.6 (5)‡
db/db	6.7 ± 0.3 (15)	135 ± 21 (10)	1.8 ± 0.2 (10)
db/db + BRL 26830	3.8 ± 0.1 (15)‡	>200	5.7 ± 1.0 (9)†
Experiment 2			
db/+	3.5 ± 0.2 (9)‡	48 ± 8 (8)†	3.8 ± 0.2 (8)‡
db/db	7.4 ± 0.6 (9)	116 ± 15 (14)	0.6 ± 0.1 (14)
db/db + BRL 26830	4.7 ± 0.4 (9)‡	279 ± 57 (10)†	3.9 ± 1.3 (10)†
db/db + Glibenclamide	6.9 ± 0.5 (9)	69 ± 5 (10)*	0.7 ± 0.2 (10)

HbA_1 was measured after 37 days of treatment in experiment 1 and after 48 days of treatment in experiment 2. The plasma insulin concentration and pancreatic insulin content were determined in mice killed after 60 (exp. 1) and 77 (exp. 2) days of treatment.
Values significantly different from *db/db* control mice: *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.001$.

treatment the values obtained were similar to those of lean *db/+* mice (Table 2). In contrast glibenclamide (50 mg/kg diet) had no significant effect on the HbA_1 level.

Plasma and pancreatic insulin. The plasma insulin concentrations of the untreated *db/db* mice were significantly increased relative to those of the lean control mice, but pancreatic insulin contents were reduced (Table 2). Treatment of the mice with BRL 26830 produced an increase in the plasma insulin concentration, but also resulted in a restoration of the pancreatic insulin content to that of the lean control mice. Glibenclamide had no effect on either the plasma concentration or pancreatic insulin content of *db/db* mice.

Food and water intake. The control *db/db* mice were hyperphagic relative to their lean littermates throughout the experimental period (Figure 3A). Neither BRL 26830 nor glibenclamide produced any consistent deviation in the food intake (results shown only for BRL 26830).

Polydipsia developed progressively in the control *db/db* mice over the first 6 wk of the experiment, and thereafter the water intake was approximately three times that of the lean mice. The development of polydipsia was completely prevented within 2 days of starting treatment with BRL 26830 (Figure 3B), but not by glibenclamide (results not shown).

In the lean mice used for the measurements of energy expenditure (see Table 4), BRL 26830 had no significant effect on either food or water intake.

Body weight and composition. Consistent with some[11] but not all[7–10] earlier findings, the body weight of the control *db/db* mice plateaued at approximately 35 g (Figure 3C). In some control *db/db* mice, there was a slight fall in body weight toward the end of the experiment, and this was exaggerated by treatment with glibenclamide. Thus, the final body weight of the glibenclamide-treated mice was significantly lower than that of the controls (control *db/db* mice 34.3 ± 0.6 g, N = 14 versus glibenclamide-treated mice 30.8 ± 0.8 g, N = 10, $P < 0.01$). In contrast, *db/db* mice given BRL 26830 continued to grow throughout the experimental period and were significantly heavier ($P < 0.001$) than were control *db/db* mice at the end of the experiments. Thus, in the first experiment, the terminal body weights (mean ± SEM) of the *db/+*, *db/db*, and BRL 26830-treated *db/db* mice were 25.7 ± 1.1 (10), 33.7 ± 1.5 (15), and 44.3 ± 0.8

TABLE 3
Acute thermogenic activity of BRL 26830 in C57Bl/KsJ *db/db* mice and their lean littermates*

Genotype	Acute treatment	Nutritional state and environmental temperature	Energy expenditure (kJ/h/mouse) over 6 h posttreatment (% increase by BRL 26830 in parentheses)
db/db	Water	Fed, 23°C	1.36 ± 0.15
db/db	BRL 26830	Fed, 23°C	1.77 ± 0.15 (130)
db/+	Water	Fed, 23°C	1.25 ± 0.05
db/+	BRL 26830	Fed, 23°C	1.82 ± 0.15 (146)†
db/db	Water	Fasted, 29°C	0.59 ± 0.08
db/db	BRL 26830	Fasted, 29°C	1.27 ± 0.01 (215)‡
db/+	Water	Fasted, 29°C	0.85 ± 0.03
db/+	BRL 26830	Fasted, 29°C	1.93 ± 0.19 (227)‡

*Female mice were obtained at age 5–6 wk and maintained on the control diet until they were 13–14 wk old. At this time, the blood glucose concentration in the *db/db* mice was 26.1 ± 1.2 mM, and was 6.1 ± 0.3 mM in the lean mice. The mice were given either BRL 26830 (10 mg/kg) or water by oral gavage, 30 min before starting measurements of energy expenditure. Results are the mean ± SEM of three pairs of mice with the percentage increase produced by BRL 26830 given in parentheses.
Significance relative to control mice of the same genotype in the same nutritional state and at the same environmental temperature: †$P < 0.05$, ‡$P < 0.01$.

TABLE 4
Effect of chronic treatment with BRL 26830 on basal metabolic rate and daily energy expenditure in *db/db* and *db/+* mice*

Genotype	Nutritional state and environmental temperature	21-h energy expenditure (kJ/h/mouse)		Treated/control
		Control	Treated	
db/db	Fed, 23°C	1.38 ± 0.08	1.86 ± 0.11	1.35
db/+	Fed, 23°C	1.46 ± 0.03	1.75 ± 0.04	1.19
db/db	Fasted, 29°C	1.09 ± 0.15	1.33 ± 0.14	1.22
db/+	Fasted, 29°C	1.05 ± 0.07	1.35 ± 0.09	1.28

*Female C57Bl/KsJ *db/+* and *db/db* mice were 13 wk old at the time of measurement and had received the experimental treatment for the preceding 6 wk. The mid-day blood glucose concentration (mM) in the mice was: control *db/db*, 26.1 ± 1.2; BRL 26830-treated *db/db*, 13.0 ± 1.9; control *db/+*, 6.1 ± 0.3; and BRL 26830-treated *db/+*, 5.9 ± 0.1.

(14), respectively. The excess weight in the BRL 26830-treated *db/db* mice was mainly lipid, but there was also an increase in the mass of several muscles (data not shown).

In the *db/+* mice used for the measurement of energy expenditure, BRL 26830 produced a small but not significant reduction in the body weight. Examination of these mice showed that there was a reduction in the size of fat pads and an increase in the weight of several muscles (data not shown).

Energy expenditure. The effects of BRL 26830 on energy expenditure were determined in separate experiments. A single acute dose of BRL 26830 (10 mg/kg), which is similar to the daily dietary intake, produced a similar percentage increase in energy expenditure in *db/db* mice as in the lean littermates. This effect was apparent whether measurements were made in fed mice at the normal animal house temperature of 23°C or in fasted mice at thermoneutrality (Table 3).

Chronic treatment of *db/db* and *db/+* mice with BRL 26830 (50 mg/kg diet) produced an increase in the 21-h energy expenditure both in fasted mice maintained at thermoneutrality (29°C) and in fed mice maintained at 23°C (Table 4).

Insulin secretion by perfused pancreas. Stimulation of the isolated, perfused pancreas from control *db/+* mice with a high concentration of glucose (16.7 mM) resulted in a biphasic release of insulin (Figure 4). The rate of basal release of insulin from the perfused pancreas of *db/db* mice was significantly higher than in the lean controls, and the biphasic release of insulin to a glucose challenge was absent. Treatment of *db/db* mice with BRL 26830 for 6 wk resulted in a partial restoration of normal insulin release kinetics (Figure 4).

DISCUSSION

BRL 26830, a β-adrenoceptor agonist (Figure 1) and structurally unrelated to any known hypoglycemic agent, was effective in lowering the mid-day blood glucose concentration in C57Bl/KsJ *db/db* mice when administered chronically as dietary admixture. This method of compound administration was chosen, since we have found that these mice do not tolerate well handling procedures such as oral gavage. At the inclusion rate of 50 mg/kg diet, the actual intake of BRL 26830 was 7.5–10 mg/kg body wt/day. Over the first 2 wk of treatment, the blood glucose concentration was reduced from 21.5 ± 0.9 to 7.1 ± 0.6 mM ($P < 0.001$) (Table 1). The mid-day blood glucose concentration was maintained at <10 mM for 40–50 days and thereafter there was a slight increase. Nevertheless, there remained a very significant hypoglycemic effect relative to untreated *db/db* mice throughout the experiment.

The blood glucose concentration of *db/db* mice is very dependent on the feeding pattern and, thus, a mid-day blood glucose measurement may not be truly representative of the overall 24-h cycle. However, 2-h measurements of blood glucose concentration over a continuous 24-h period indicated that there were no hyperglycemic episodes in the *db/db* mice given BRL 26830.

The long-term efficacy of BRL 26830 in achieving improved blood glucose control was apparent from the decrease in glycosylated hemoglobin concentration and the lowered water intake. Urinary glucose output was not quantified in the present study, but the normalization of water intake (and presumably urine volume) is interpreted as indicating that there is at least a substantial reduction in glucose excretion.

In addition to its effect on blood glucose, BRL 26830 restored the pancreatic insulin content of *db/db* mice (Table 2) to that found in lean mice and also partially restored the first-phase insulin secretagogue response to a glucose challenge (Figure 4). These changes could be due to an alleviation of the demand for insulin caused by improved glucose clearance. However, the finding that the plasma insulin concentration was also increased suggests that either the total amount of insulin produced by the pancreas increases after treatment or that insulin clearance is decreased. It has been suggested, as a result of studies in both Chinese hamsters and in human non-insulin-dependent diabetes, that normal-

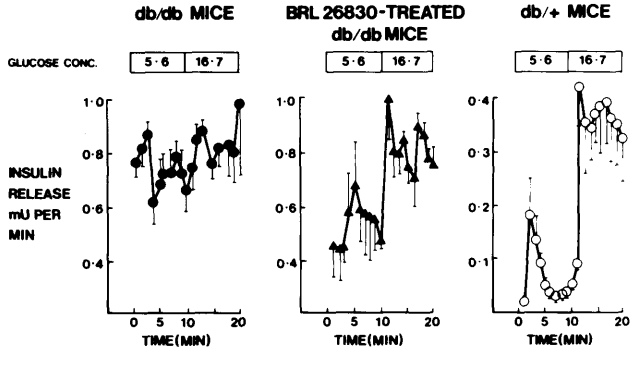

FIGURE 4. The effect of chronic treatment of *db/db* mice with BRL 26830 on glucose-stimulated insulin secretion by the isolated, perfused pancreas. BRL 26830 was admixed with food at 50 mg/kg concentration and was supplied to 5-wk-old *db/db* mice for 3–5 wk. The results are given as mean ± SEM. There were four perfusions from the control *db/db* mice, five perfusions from the BRL 26830-treated mice, and four perfusions from the *db/+* mice.

ization of plasma glucose levels is by itself sufficient to partially restore insulin secretion to a glucose challenge.[21,22] Studies to determine the daily rate of C-peptide secretion are required to resolve this point.

BRL 26830 had no overall effect on food consumption in *db/db* mice. However, over the first 2 wk of treatment there was a small but significant increase in food intake. These findings contrast with recent results obtained with ciglitazone,[11] which tends to decrease food intake, and demonstrate also that the hypoglycemic action of BRL 26830 is not a consequence of a reduction in dietary intake.[7]

The finding that BRL 26830 produced an increase in the growth rate and final body weight of the *db/db* mice is at first sight paradoxical, since BRL 26830 has been demonstrated previously to have potent thermogenic and antiobesity activity in a number of animal models,[12] including *ob/ob* mice. Furthermore, current data (Table 3) show unequivocally that BRL 26830 has the capability of activating thermogenic mechanisms in *db/db* mice when given acutely by oral gavage. In considering this apparent paradox, there are a number of factors that must be considered. First, it should be noted that the growth rate of the control *db/db* mice used in the present experiment plateaued at approximately 7–8 wk of age at a body weight of 30–35 g. Thus, although *db/db* mice are known to be energetically highly efficient during early life,[23,24] this does not pertain to the *db/db* mice used in the present study. Second, it appears that different colonies of *db/db* mice, even when they have the same genetic background, show a plateau in growth rate at different ages. Thus, in the studies of Chang et al.,[11] who also obtained their mice directly from Jackson Laboratories, the plateau body weight was 30–35 g. However, other studies have shown linear growth rates up to 50 g.[7,9,10,25,26] In the main, this additional weight is lipid. It is probable that these differences in maximum body size reflect varying degrees of pancreatic insulin secretagogue insufficiency coupled with marked resistance to insulin action in peripheral tissues. Thus, in the present studies and in those of Chang,[11] the earlier onset of overt diabetes in control mice has resulted in a premature arrestment of growth. Significantly, in both studies, improvement in diabetic status after treatment either with BRL 26830 or ciglitazone[11] was accompanied by a restoration of growth. Thus, over a 58-day treatment period, ciglitazone, which has no thermogenic activity in vivo (our unpublished results), produced a 20-g increase in body weight.[11] In the present study, treatment for a slightly longer period with BRL 26830 produced only an 11-g increase. It seems likely that this difference in growth rate reflects the antiobesity activity of BRL 26830.

Data shown in Table 4 indicate that, on a per mouse basis, chronic treatment of *db/db* mice with BRL 26830 leads to an increase in energy expenditure. However, it is not possible from these data to identify directly whether the increase seen is a direct result of the thermogenic activity of BRL 26830 or an indirect effect simply because the BRL 26830-treated *db/db* mice are larger and are no longer diabetic. Comparison of results obtained in fed mice at an environmental temperature of 23°C with those obtained in fasted mice at 29°C shows that the increase in dietary and thermoregulatory thermogenesis is significantly higher ($P < 0.05$) in the BRL 26830-treated *db/db* mice (0.53 kJ/h/mouse) than in controls (0.29 kJ/h/mouse). The ratio of these values (1.83) is substantially greater than the ratio of the basal metabolic rates of the two sets of mice (1.22), and this suggests that chronic treatment of the mice with BRL 26830 does have a direct effect on the dietary and thermoregulatory component of metabolic rate.

The efficacy of BRL 26830 as an antidiabetic agent in *db/db* mice is in marked contrast to the sulfonylureas. In the present study it has been shown that glibenclamide, a second-generation sulfonylurea, was totally ineffective in altering any of the measured parameters of diabetic status, and these findings are in agreement with those of earlier studies[27,28] that used tolbutamide. Such differences in efficacy suggest that there are mechanistic differences in mode of action of BRL 26830 and sulfonylureas. Sulfonylureas have been claimed to have extrapancreatic activity,[28–31] as well as insulin secretagogue activity, although the former effects have not been demonstrated in *db/db* mice. BRL 26830 has also been shown to have insulin secretagogue activity in normal rats and to improve insulin sensitivity in obese C57Bl/6 *ob/ob* mice and Zucker fa/fa rats,[13] which could result in both increased peripheral utilization and decreased hepatic glucose production. However, BRL 26830 is also able to activate the thermogenic pathway in brown adipose tissue of rodents.[12,32] In recent studies,[33] we have shown that the maximum capacity of the glycolytic pathway in brown adipose tissue is reduced in *db/db* mice relative to that in *db/+* mice, and that treatment of *db/db* mice with BRL 26830 specifically increased by 10-fold the maximum activity of hexokinase in brown adipose tissue. Moreover, measurements of whole body 2-deoxyglucose uptake indicated an increased glucose uptake by brown adipose tissue of *db/db* mice given BRL 26830. These findings raise the possibility that the antidiabetic and thermogenic activities of BRL 26830 in *db/db* mice are linked, and that blood glucose may be either a direct substrate for thermogenesis (via glycolytically produced pyruvate) or an indirect substrate via its role as a lipogenic precursor. The relevance of such a mechanism of action to maturity-onset diabetes in man is not known, but is currently under investigation.

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