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ANTI-HYPERGLYCAEMIC ACTION OF BRL 26830, A NOVEL β-ADRENOCEPTOR AGONIST, IN MICE AND RATS†

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Abstract—BRL 26830, (R*,R*)-(±)-methyl 4-[2-[(2-hydroxy-2-phenylethyl)amino] propyl] benzoate, is a new orally active anti-hyperglycaemic agent. In 24 hr-fasted rats and mice, BRL 26830 decreased the blood glucose concentration following the administration of a subcutaneous glucose load. It also improved oral and intravenous glucose tolerance in 24 hr-fasted rats and decreased the post-prandial blood glucose concentration following the consumption of the complete, milk-based, meal "Nutrament". BRL 26830 produced a dose-related increase in the plasma insulin concentration and since it was inactive in lowering blood glucose in streptozotocin-diabetic rats, it is likely that its acute action on glucose tolerance was through the stimulation of insulin secretion. In contrast to the sulphonylurea, glibenclamide, BRL 26830 had no effect on the blood glucose concentration in 5 hr-fasted rats and only produced a transient reduction in 24 hr-fasted rats.

BRL 26830 did not improve glucose tolerance when given acutely to hyperinsulinaemic C57BL/6 *ob/ob* mice. However, chronic treatment of these mice with BRL 26830 for 14–43 days resulted in a significant improvement in glucose tolerance.

Type II, non-insulin dependent, diabetes mellitus is characterized by defects in both insulin secretion and insulin action [1–4]. Impaired pancreatic islet-cell function is invariably associated with an absence of a first-phase insulin response to a glucose stimulus but may also involve decreased sensitivity of insulin responses to glucose although sensitivity to other secretagogues can be normal [5, 6]. In severe type II diabetes, there may be a decrease in the overall insulin secretory capacity [3–7]. Resistance to the action of insulin can involve both a reduction in the number of insulin receptors and post-insulin receptor defects [8]. It is not defined whether defective secretion or defective action of insulin is the fundamental defect in type II diabetes; if indeed there is a single defect. Nevertheless, there is a growing view that the successful treatment of type II diabetes will require improvements in both the insulin secretagogue capacity and the alleviation of insulin resistance.

The most commonly used oral anti-diabetic drugs are the sulphonylureas. These agents both stimulate insulin secretion [9, 10] and increase the number of insulin receptors in peripheral tissues [11–13]. Recently, evidence has been presented suggesting that sulphonylurea therapy may result also in the correction of post-receptor defects in insulin action [14]. The sulphonylureas have two major drawbacks. First, they tend to encourage weight gain [15] and, since obesity invariably leads to a decrease in the insulin sensitivity [16], any weight gain induced by sulphonylurea therapy tends to reduce the clinical effectiveness of the treatment [17]. The second and more serious drawback is that they can produce profound hypoglycaemia in some subjects [17, 18].

The present paper describes the pharmacological profile of a new anti-hyperglycaemic agent, which is structurally unrelated to the clinically-used anti-diabetic drugs. BRL 26830, (R*,R*)-(±)-methyl 4-[2-[(2-hydroxy-2-phenylethyl)amino]propyl] benzoate, (E)-2-butenedioate (2:1) salt [19], is a β-adrenoceptor agonist with a novel type of β-receptor selectivity [20, 21] that may find clinical utility in the treatment of both obesity and type II diabetes. In common with the sulphonylureas, BRL 26830 is an insulin secretagogue and produces improvements in glucose tolerance in previously insulin-resistant, glucose-intolerant animals. In contrast to the sulphonylureas, it activates counter-regulatory systems, thus preventing hypoglycaemia. In addition to its effects on glucose metabolism, BRL 26830 has thermogenic properties that result in a reduction in the adiposity of obese animals [19].

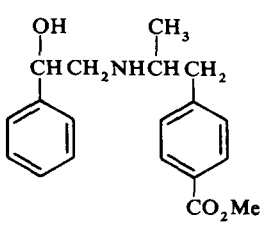

Fig. 1. Structure of BRL 26830.

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MATERIALS AND METHODS

Animals. Female CFLP mice (20–25 g) were obtained from Hacking and Churchill, Huntingdon, Cambs. Male Sprague–Dawley rats (200–250 g) were supplied by T. Tuck, Battlebridge, Essex.

C57BL/6 male genetically obese (*ob/ob*) mice were obtained from Olac 1976 Ltd., Bicester, Oxon. The *ob/ob* mice were 9/10 weeks-old at the start of the studies.

Diabetes was induced by the intravenous injection of streptozotocin (65 mg/kg) to fed rats. All animals were allowed food (Oxoid Breeders diet, H.C. Styles, Bewdley, Worcs) and water *ad lib.* and were kept under a controlled light cycle (lights on 0600–1800 hr) at a temperature of 23 ± 1°.

Hypoglycaemic agents. The original synthesis of BRL 26830 (Fig. 1) was carried out in these laboratories, and will be reported elsewhere. All studies in the present paper have used BRL 26830 as the hemifumarate salt.

We are indebted to Pfizer Ltd., Sandwich, U.K. for the gifts of chlorpropamide and glipizide. Glibenclamide was a gift from Roussel Ltd., London.

Glucose tolerance tests. For glucose tolerance tests in normoglycaemic mice, BRL 26830 was given by oral gavage 30 min prior to a subcutaneous injection or oral load of 1 g glucose/kg body wt. Serial blood samples (10 μl) were obtained from the cut tip of the tail at 30-min intervals for the measurement of blood glucose concentration. A similar procedure was adopted in rats except that the glucose load used for both subcutaneous and oral glucose tolerance tests was 1.2 g/kg body wt.

Meal tolerance test. Normoglycaemic rats were fasted for 24 hr prior to receiving BRL 26830 or water (control vehicle) orally. Thirty minutes later, the rats were given 10 ml/kg body weight of Nutrament (Mead Johnson, Division of Bristol-Myers Co. Ltd., S. Ruislip, Middlesex) by oral gavage. This substance is a milk-based liquid meal, which supplies 5.4 g protein, 3.6 g fat and 14.1 g carbohydrate per 100 ml. Its calorific value is 110 kcal/100 ml. Blood samples for the measurement of the glucose concentration were obtained as described earlier.

Repeat-dose studies. Male *ob/ob* mice were dosed orally with water or BRL 26830 (1 mg/kg) once a day for 21 days. The mice were weighed weekly. After the treatment period, mice were fasted for 24 hr. The mice were then given their appropriate drug treatment followed 30 min later by a subcutaneous injection of glucose (0.6 g/kg body wt). This dose of glucose was chosen to be approximately equivalent on a fat-free body mass basis to that used in normoglycaemic mice.

Insulin secretion studies. Male Sprague–Dawley rats were anaesthetized with Na pentobarbitone (60 mg/kg i.p.). The carotid artery was exposed and fitted with a cannula, which was maintained patent with heparin/saline. After taking an initial blood sample (1 ml), the rats were injected intraperitoneally with either saline (vehicle) or BRL 26830. Further blood samples were obtained after 5, 30 and 60 min. Plasma samples were stored at −20° until assayed for insulin.

Analytical procedures. Blood glucose was determined either by the glucose oxidase assay using a Technicon autoanalyzer II [22] or by the hexokinase method [23] (Boehringer, Lewes, Sussex) using a Clinicon Corona kinetic analyzer. The area under the glucose tolerance curve was calculated trigonometrically. Insulin was determined in plasma samples by a double-antibody procedure [24] using human insulin as a standard. The insulin-binding reagent was obtained from Wellcome Laboratories, (Beckenham, Kent) and the I^{125}-insulin from the Radiochemical Centre, (Amersham, U.K.). Results are expressed as μ units human insulin equivalents per ml of plasma.

Statistics. All values are given as mean ± S.D. Statistical significance between control and each treated group was tested using the Student's *t*-test.

RESULTS

Anti-hyperglycaemic activity in non-diabetic mice and rats

The glucose tolerance test in 24 hr-fasted mice was used as the primary test for detecting the anti-hyperglycaemic activity of BRL 26830. To avoid flattening of blood glucose curves as a result of drug effects on stomach emptying and gastrointestinal absorption of glucose, the glucose load was given subcutaneously. As shown in Table 1, BRL 26830 produced a decrease in fasting blood glucose and an increase in glucose tolerance over the dose range 0.08–2.0 mg/kg p.o. The potency of BRL 26830 in this test was similar to that of the second generation sulphonylureas, glibenclamide and glipizide, and it was more potent that the first generation sulphonylureas such as tolbutamide and chlorpropamide (Fig. 2). BRL 26830 showed a similar level of potency in improving both oral and subcutaneous glucose tolerance (results not shown).

In the subcutaneous glucose tolerance test in 24 hr-fasted rats, BRL 26830, over the dose-range 1–10 mg/kg p.o., produced a dose-related reduction in the area under the glucose tolerance curve (results not shown). The absolute potency of BRL 26830 in this test was between that of the first and second

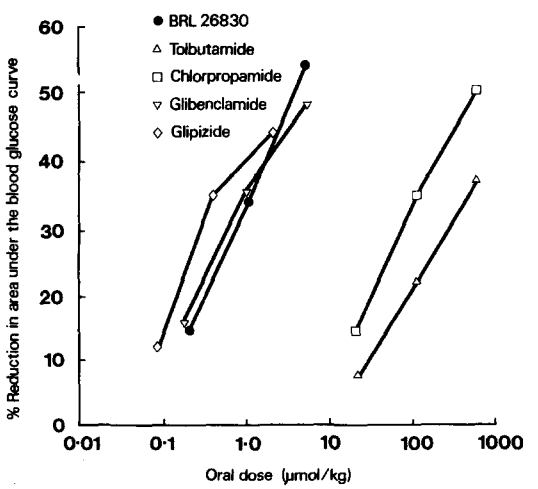

Fig. 2. Dose–response of BRL 26830 and sulphonylureas on subcutaneous glucose tolerance in mice.

Table 1. Effect of BRL 26830 on subcutaneous glucose tolerance in 24 hr-fasted mice

Treatment given 30 min prior to glucose load	Blood glucose (mM) at time after glucose load (min) 0	30	60	90	120	(%) reduction in area under blood glucose curve relative to water-dosed controls
Water 10 ml/kg	3.3 ± 0.2	8.3 ± 1.7	6.3 ± 1.4	4.6 ± 0.9	4.1 ± 0.5	—
BRL 26830 0.08 mg/kg	2.5 ± 0.5†	6.9 ± 0.4	5.7 ± 1.1	4.1 ± 0.5	3.8 ± 0.3	14
BRL 26830 0.4 mg/kg	2.0 ± 0.2‡	5.0 ± 0.9†	4.2 ± 0.8*	3.1 ± 0.9*	3.4 ± 0.6	34
BRL 26830 2.0 mg/kg	2.1 ± 0.1‡	3.5 ± 0.5‡	2.3 ± 0.3‡	1.9 ± 0.3‡	2.2 ± 0.4‡	54

Results are mean ± S.D., N = 5
Significance compared to water dosed control at same time-point.
* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

generation sulphonylureas (Fig. 3). In order to estimate the efficacy of BRL 26830 in reducing post-prandial hyperglycaemia, we have measured the blood glucose concentration following oral administration of the complete, liquid, milk-based meal, "Nutrament". As shown in Table 2, BRL 26830, given orally 30 min prior to the meal, was effective in reducing the post-prandial glucose surge.

BRL 26830 was effective also in improving intravenous glucose tolerance in 24 hr-fasted rats (glucose load 1.2 g/kg). Thus, at a dose level of 5 mg/kg p.o., BRL 26830 produced an increase in the fractional glucose disposal rate (1.18 ± 0.07% per min in BRL 26830-treated rats; 0.76 ± 0.07 in control rats; $P < 0.01$). Glibenclamide (2 mg/kg p.o.) increased the disposal rate to 1.10 ± 0.07% per min ($P < 0.01$).

In rats fasted for only 5 hr, BRL 26830 (8 mg/kg p.o.) did not produce any alteration in the tolerance to either subcutaneous or oral glucose loads (results not shown).

Activity in hyperglycaemic, hyperinsulinaemic mice

C57BL/6 *ob/ob* mice are hyperinsulinaemic. Although they generally have fasting blood glucose concentrations within the normal range, they show abnormal glucose tolerance, which is due principally to their extreme insulin resistance [25]. In contrast to the findings in lean normoglycaemic mice, a single oral dose of BRL 26830 (1 mg/kg) had no beneficial effect on subcutaneous glucose tolerance in the ob/ob mice (Table 3). Indeed, in some studies glucose intolerance was slightly exacerbated by acute treatment with BRL 26830. However, daily treatment of C57BL/6 *ob/ob* mice with BRL 26830 (1 mg/kg p.o.) for periods of 14–43 days consistently led to improved glucose tolerance (Table 4). In these studies, there was no indication of development of pharmacological tolerance, but in order to address this question specifically, mice were treated daily with either BRL 26830 (1 mg/kg p.o.) for a 6 week period or with water for the first three weeks followed by BRL 26830 (1 mg/kg p.o.) for the final 3 weeks. The blood glucose profiles following a subcutaneous glucose tolerance test were virtually identical in both treatment groups and were improved significantly relative to the glucose profile of the control group (Table 4).

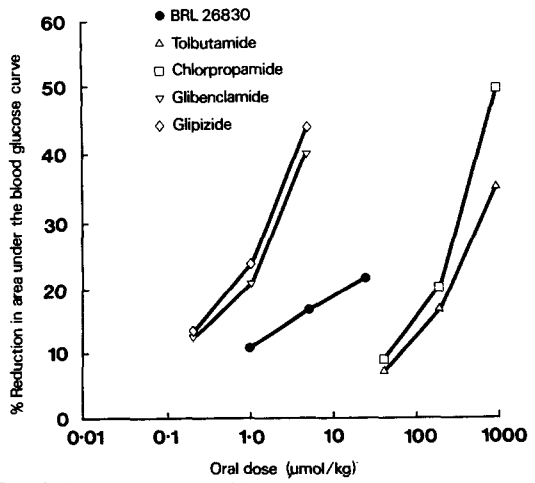

Fig. 3. Dose–response of BRL 26830 and sulphonylureas on subcutaneous glucose tolerance in rats.

In previous studies [19] it has been shown that BRL 26830 has thermogenic activity, which in obese mice including C57BL/6 *ob/ob* mice leads to a reduction in the degree of adiposity. Since a reduction in the obesity of the mice would be expected to reduce the degree of insulin resistance, it is important to note that in the studies described in Table 4 the dose level of BRL 26830 used was below that which produces a significant reduction in body weight over the time course of the experiment.

To ascertain that the anti-hyperglycaemic activity produced by BRL 26830 in C57BL/6 *ob/ob* mice is not a property common to all β-adrenoceptor agonists, we have examined the effect of salbutamol and fenoterol in this model. Both of these β_2-adrenoceptor agonists have thermogenic activity [26, 27]. Fenoterol (5 mg/kg p.o.) given daily for 21 days had no significant effect on glucose tolerance in this model whereas salbutamol (20 mg/kg p.o.) exacerbated the glucose intolerance (Table 5).

Activity in streptozotocin-diabetic rats

BRL 26830 (5 mg/kg), given either acutely or by daily oral gavage for 14 days, had no effect on the

Table 2. Effect of BRL 26830 on the blood glucose surge produced by an oral dose of Nutrament in 24 hr-fasted rats

Treatment given 30 min prior to Nutrament Load		Blood glucose (mM) at time (min) after dosing nutrament 0	30	60	90	120	180	240	(%) reduction in area under blood glucose curve
Water	1 ml/kg	4.5 ± 0.3	6.2 ± 1.1	5.6 ± 0.8	5.7 ± 0.7	5.7 ± 0.5	4.6 ± 0.7	4.6 ± 0.2	
BRL 26380	1 mg/kg	4.3 ± 0.4	5.3 ± 0.4	5.3 ± 0.6	4.9 ± 0.2	5.5 ± 0.1	3.9 ± 0.6	3.9 ± 0.7	9
BRL 26830	2 mg/kg	4.2 ± 0.2	5.4 ± 0.3	5.1 ± 0.5	4.9 ± 0.3	4.6 ± 0.7*	4.0 ± 0.3	4.0 ± 0.3†	13
BRL 26830	4 mg/kg	4.3 ± 0.2	5.3 ± 0.5	4.5 ± 0.4	4.6 ± 0.3*	4.7 ± 0.4*	3.7 ± 0.3*	3.5 ± 0.6†	16
BRL 26830	8 mg/kg	4.2 ± 0.8	4.4 ± 0.7*	4.1 ± 0.4†	4.4 ± 0.3†	4.6 ± 0.5†	4.0 ± 0.4	3.5 ± 0.7*	19

Results are the mean ± S.D. of 5 values.
Significance compared to water-dosed control at same time-point. * $P < 0.05$, † $P < 0.01$.

Table 3. Glucose tolerance in 24 hr-fasted C57B1/6 *ob/ob* male mice given a single dose of BRL 26830*

Time relative to glucose load (min)	Blood glucose (mM) Control	BRL 26830 (1 mg/kg p.o.)
−30	5.5 ± 0.6	8.4 ± 2.6
0	8.2 ± 1.9	10.1 ± 2.8
30	11.7 ± 1.7	12.3 ± 3.4
60	9.6 ± 1.5	9.7 ± 3.6
120	5.8 ± 1.3	7.0 ± 3.7
240	4.3 ± 0.9	4.5 ± 2.4

* BRL 26830 was given 30 min prior to the subcutaneous glucose load.
Results are the mean ± S.D. of values from 8 mice.

blood glucose concentration of 5 hr-fasted streptozotocin-diabetic rats. In such rats, glibenclamide (1 mg/kg p.o.) was also ineffective (results not shown).

Insulin secretagogue activity in normal rats

It is well established that the rat pancreas possesses β-adrenoreceptors, stimulation of which leads to the release of insulin. Isoproterenol is a potent insulin secretagogue, whose action can be demonstrated under both normoglycaemic and hyperglycaemic conditions [7]. The insulin secretagogue effect of BRL 26830 was evaluated in normoglycaemic, anaesthetized rats. It was a potent insulin secratagogue, in both 24 hr-fasted rats (Table 6) and in 5 hr-fasted rats, with a rapid onset of action.

Effects on fasting blood glucose in normoglycaemic rats and mice

As part of the glucose tolerance tests, blood samples were taken routinely 30 min after the administration of BRL 26830, but before the administration of glucose. In these experiments, which used 24 hr-fasted mice or rats, treatment with BRL 26830 resulted invariably in a small fall in the blood glucose concentration over the 30 min period prior to the glucose load. However, in spite of using dose levels up to ten times the effective dose, BRL 26830 did not produce frank hypoglycaemia. In more extensive studies, the relative effects of BRL 26830 and glibenclamide on blood glucose concentrations were determined in both 24 hr- and 5 hr-fasted rats. In the 5 hr-fasted rats, BRL 26830, even at the very high dose level of 186 mg/kg, had no significant effect on the blood glucose concentration, whereas glibenclamide at a dose of only 5 mg/kg p.o. produced a significant decrease (Table 7). In 5 hr-fasted rats, dosage with BRL 26830 (8 mg/kg) produced a decrease in the liver glycogen content from 102 ± 5 μmole glycogen-glucose units/g to 56 ± 9 μmole glycogen-glucose units/g. Thus, at least in part, the maintenance of normoglycaemia in 5 hr-fasted rats is achieved by a mobilization of liver glycogen. However, in 24 hr-fasted rats, which have low reserves of liver glycogen, BRL 26830 still did not induce frank hypoglycaemia (Table 7).

Table 4. Effect of chronic treatment with BRL 26830 on glucose tolerance in C57BL/6 *ob/ob* mice*

Time relative to glucose load (min)	Blood glucose concentration (mM)		
	Water: day 1–42 (N = 10)	Water: day 1–21 BRL 26830: day 22–42 (N = 9)	BRL 26830: day 1–42 (N = 9)
−30	6.8 ± 2.5	4.8 ± 0.6†	4.7 ± 1.0
0	7.4 ± 3.7	6.6 ± 0.9	5.9 ± 0.8
+30	11.2 ± 4.0	7.4 ± 0.9†	7.1 ± 0.8‡
+60	9.2 ± 4.9	4.5 ± 0.3†	4.8 ± 0.6†
+120	6.5 ± 3.5	3.9 ± 0.3†	4.0 ± 0.5
+240	4.3 ± 1.7	3.6 ± 0.6	4.0 ± 0.5
Reduction in area under blood glucose curve (%)	—	34	33

* Male C57BL/6 *ob/ob* mice were dosed once daily with water (10 ml/kg p.o.) or an aqueous solution of BRL 26830 (1.0 mg/kg p.o.). The glucose tolerance test was carried out on the 42nd day of treatment following a 24 hr-fast. On the 42nd day, the treatments were given as usual and 30 min later each mouse received glucose (0.6 g/kg s.c.). The body weights (g) of the three groups of mice at the end of the treatment were 46.6 ± 3.2, 46.8 ± 1.9 and 44.8 ± 4.4, respectively. The weight gains (g) during the 42-day treatment period for each treatment group were 11.9 ± 2.0, 12.4 ± 1.6 and 9.9 ± 1.4, respectively.

Results are given as the mean ± S.D. of the blood glucose concentration.

Significance compared to water-dosed controls at same time-point. † $P < 0.05$, ‡ $P < 0.01$.

Table 5. Effect of chronic treatment with salbutamol or fenoterol on glucose tolerance in genetically (*ob/ob*) obese mice*

Time relative glucose load (min)	Blood glucose (mM)		
	Control	Salbutamol	Fenoterol
−30	4.5 ± 0.8	4.4 ± 0.6	3.6 ± 0.7
0	7.1 ± 3.2	16.9 ± 0.9†	9.2 ± 1.6
+30	11.4 ± 4.3	18.7 ± 1.4‡	12.5 ± 1.9
+60	9.3 ± 3.3	15.4 ± 1.4‡	9.0 ± 2.7
+120	8.3 ± 4.7	10.1 ± 1.6	6.8 ± 1.6
+240	5.7 ± 0.6	5.7 ± 0.6	4.4 ± 0.8
Reduction in area under curve	—	−41%	7%

* Male C57BL/6 mice, aged 10–11 weeks, were treated daily with water, salbutamol (20 mg/kg p.o.) or fenoterol (5 mg/kg p.o.) daily for 20 days. The mice were then fasted for 24 hr prior to a subcutaneous glucose tolerance test. Results are the mean ± S.D. of 10 mice.

Significant differences from controls are indicated as follows: † $P < 0.001$, ‡ $P < 0.01$.

Table 6. Acute effect of BRL 26830 on plasma insulin concentration in 24 hr-fasted anaesthetized rats

Treatment and time after treatment (min)		Plasma immunoreactive insulin (μ units human equivalent/ml)			
		0	5	30	60
Control		36 ± 24	25 ± 18	21 ± 14	28 ± 14
BRL 26830	(0.4 mg/kg)	37 ± 17	45 ± 30	53 ± 21*	31 ± 9
BRL 26830	(1.0 mg/kg)	37 ± 12	41 ± 17	138 ± 46‡	70 ± 18†
BRL 26830	(2.0 mg/kg)	26 ± 9	45 ± 9	206 ± 106†	106 ± 83
BRL 26830	(4.0 mg/kg)	39 ± 12	70 ± 63	310 ± 164†	200 ± 145*
BRL 26830	(8.0 mg/kg)	25 ± 15	53 ± 22	246 ± 89‡	153 ± 77†
BRL 26830	(40 mg/kg)	32 ± 6	86 ± 67	314 ± 162†	313 ± 190*

The values are given as the mean ± S.D. of 5 values.

* Significantly different from saline-injected control at the same time-point, $P < 0.05$.

† Significantly different from saline-injected control at the same time-point, $P < 0.01$.

‡ Significantly different from saline-injected control at the same time-point, $P < 0.001$.

Table 7. Effect of Glibenclamide and BRL 26830 on fasting blood glucose in 24 hr and 5 hr-fasted rats

		Time relative to administration of compounds (min) 0	30	60	120	180	240
5 hr-fasted							
Control		4.9 ± 0.2	5.6 ± 0.2	5.2 ± 0.7	4.6 ± 0.8	4.6 ± 0.5	4.4 ± 0.6
BRL 26830	186 mg/kg	5.3 ± 0.5	6.4 ± 0.5*	5.3 ± 0.6	4.7 ± 0.4	4.8 ± 0.6	4.7 ± 0.5
BRL 26830	40 mg/kg	5.2 ± 1.0	5.7 ± 0.9	5.3 ± 0.6	4.6 ± 0.2	4.6 ± 0.2	4.6 ± 0.6
Glibenclamide	25 mg/kg	5.2 ± 0.3	3.8 ± 1.1†	3.0 ± 0.4†	2.5 ± 0.3†	2.8 ± 0.5†	2.6 ± 0.3
Glibenclamide	5 mg/kg	4.8 ± 0.7	5.0 ± 0.6	2.9 ± 0.5†	2.6 ± 0.3†	2.9 ± 0.3†	2.8 ± 0.7†
24 hr-fasted							
Control		3.7 ± 0.3	4.7 ± 0.5	4.1 ± 0.4	3.7 ± 0.4	2.8 ± 0.9	2.8 ± 0.7
BRL 26830	186 mg/kg	3.7 ± 0.3	4.7 ± 0.6	3.4 ± 0.5*	2.8 ± 0.4	2.6 ± 0.5	2.5 ± 0.6
BRL 26830	40 mg/kg	3.6 ± 0.7	4.2 ± 0.8	2.7 ± 0.6†	2.3 ± 0.4†	2.2 ± 0.5	2.4 ± 0.6
Glibenclamide	25 mg/kg	3.2 ± 0.5	2.7 ± 0.4‡	2.0 ± 0.5‡	1.7 ± 0.4‡	1.9 ± 0.4	1.7 ± 0.3*
Glibenclamide	5 mg/kg	3.5 ± 0.7	3.7 ± 1.1	2.2 ± 0.5‡	1.8 ± 0.3‡	1.8 ± 0.3	1.4 ± 0.2†

Significantly different from controls at the same time-point, * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

DISCUSSION

BRL 26830 is a representative of a novel series of anti-hyperglycaemic agents that are structurally unrelated to currently available oral hypoglycaemic drugs. In glucose tolerance tests, BRL 26830 is more potent in 24 hr-fasted mice than in rats (Figs 2 and 3) but in both species, the activity of the compound is rapid in onset. The rapid absorption of BRL 26830 has been confirmed in the rat by radiochemical studies. These studies have shown also that BRL 26830 is rapidly de-esterified *in vivo* (P. J. Baines, A. J. Swaisland and G. Mellows, unpublished observations) and it is probably the free acid (BRL 28410) that mediates the activities of BRL 26830 *in vivo*.

Similar dose–response curves were obtained for BRL 26830 in oral and subcutaneous glucose tolerance studies in both rats and mice. Thus, the effect of BRL 26830 on glucose tolerance cannot be due simply to inhibition of glucose absorption from the gut. The possibility that BRL 26830 may act by stimulation of insulin secretion is indicated by the finding that it was inactive in lowering blood glucose in streptozotocin-diabetic rats and that it produced a dose-related increase in the plasma immunoreactive insulin concentration (Table 6).

In spite of its potent effect in raising the plasma insulin concentration in 24 hr-fasted rats, BRL 26830 (1–8 mg/kg) had no effect on the fasting blood glucose concentration (Table 2, zero time value is taken 30 min after administration of BRL 26830). A possible explanation for this apparent paradox is that BRL 26830 increased both the rate of glucose utilization (through its insulin secretagogue action) and the rate of endogenous glucose production. Thus, even in those situations where BRL 26830 is not affecting blood glucose concentration, it is proposed that glucose turnover is increased. Several lines of evidence lend support to this proposal. BRL 26830 improves glucose tolerance only in 24 hr-fasted rats and not in 5 hr-fasted rats. In both nutritional states, BRL 26830 (5 mg/kg) produced a similar increase in plasma insulin concentration. A major difference between the states is the hepatic glycogen content. In 5 hr-fasted rats it is about 100 μmoles glycogen-glucose units/g liver whereas in 24 hr-fasted rats we routinely find the values of less than 20 μmoles glycogen-glucose units/g liver. The administration of BRL 26830 to 5 hr-fasted rats resulted in a 50% fall in the hepatic glycogen content. This is equivalent of 40% of the administered glucose load. Thus, although there is no change in the blood glucose concentration during a glucose tolerance test in 5 hr-fasted rats, the calculated glucose disposal rate of BRL 26830-treated rats was increased by at least 40%.

The counter-regulatory effects are also seen in experiments in which the response of fasting blood glucose to large doses (40 and 186 mg/kg p.o.) of BRL 26830 was determined. In 5 hr-fasted rats, there was no fall in the blood glucose concentration up to 4 hr post-dosing. Even in 24 hr-fasted rats, the blood glucose concentration 4 hr after dosing was not significantly different from controls (Table 7), although there were significant reductions in blood glucose at intermediate time points. Glibenclamide (5 and 25 mg/kg), in contrast, produced profound reductions in the fasting blood glucose concentration.

We conclude that the effect of BRL 26830 on blood glucose concentration in normoglycaemic rats and mice is dependent on the nutritional state of the animal. It is only in those animals with depleted hepatic glycogen stores than an effect on blood glucose concentration is seen. However, even when there is no change in blood glucose concentration, the rates of glucose disposal and hence glucose turnover are increased [28]. We believe that this is the first time that a drug treatment has been suggested to increase glucose turnover without affecting blood glucose levels. However, in a recent abstract [29], it was shown that in infusion of a cocktail of insulin, glucagon, epinephrine and cortisol to a normal man produced an increased glucose turnover under euglycaemic clamp conditions.

The effects of BRL 26830 in C57BL/6 *ob/ob* mice are also consistent with the compound affecting both glucose disposal and glucose production. These animals are known to be hyperinsulinaemic and insulin resistant [25]. The administration of a single dose of BRL 26830 to these animals had either no effect on glucose tolerance (Table 3) or produced a small

exacerbation of the glucose intolerance. It seems likely that the insulin resistance of these mice limits the effects of BRL 26830 on insulin-mediated glucose utilization. In such circumstances, an increased rate of glucose production may exacerbate the glucose intolerance. However, chronic treatment of C57BL/6 *ob/ob* mice for periods of 14–42 days has consistently led to a marked improvement in glucose tolerance (Table 4). It is suggested that this effect arises principally from an increase in the insulin sensitivity of these mice. Consistent with this view is the finding of a decrease in the fasting plasma insulin concentration from 800 μU/ml in age-matched control *ob/ob* mice to 294 ± 107 μU/ml in mice that had been treated chronically with BRL 26830 for 3 weeks.

BRL 26830 is thermogenic and has been shown to produce a reduction in the body lipid content of C57BL/6 *ob/ob* mice [19]. Such an effect on body weight in the present experiment could be the basis for the improvement in glucose tolerance. However, the dose level used in the present experiment was 1 mg/kg p.o. daily whereas that used in the anti-obesity experiments was approx. 5 mg/kg p.o. per day. At the dose level of 1 mg/kg p.o. there was no significant reduction in body weight even over a 42-day period. Thus, it is unlikely that the effect of BRL 26830 on glucose tolerance in C57BL/6 *ob/ob* mice is merely a secondary consequence of the anti-obesity action.

BRL 26830 is chemically related to some β-adrenoceptor agonists. Studies *in vitro* have shown that it has β-agonist activity in a number of biological systems, but it has a different selectivity to currently available β_1 and β_2-agonists. BRL 28410, the active metabolite of BRL 26830, is a selective agonist for the brown [21] and white adipose tissue lipolytic β-adrenoceptors [20] rather than for the receptors that mediate atrial rate or bronchus smooth muscle contraction.

β-Adrenoceptor agonists are known to affect the regulation of blood glucose levels by various mechanisms. β-Agonists stimulate both insulin [30, 31] and glucagon [32, 33] release from the pancreas, and inhibit insulin-mediated glucose uptake by muscle [34–36]. In the rat, isoproterenol and the β_2-selective agonists such as salbutamol, elevate fasting blood glucose [37, 38] and exacerbate glucose tolerance (our unpublished observations). The finding that BRL 26830 can improve glucose tolerance and does not elevate fasting blood glucose in normal rats suggests that, if its effects are through β-adrenoceptor stimulation, then BRL 26830 (or BRL 28410) shows different selectivity to either isoproterenol or salbutamol with respect to those β-adrenoceptors involved in blood glucose regulation.

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