Phenylalkyloxirane Carboxylic Acids, a New Class of Hypoglycaemic Substances: Hypoglycaemic and Hypoketonaemic Effects of Sodium 2-[5-(4-Chlorophenyl)-pentyl]-oxirane-2-carboxylate (B 807–27) in Fasted Animals

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Summary. Phenylalkyloxirane carboxylic acids and esters are a new class of potent hypoglycaemic substances. Sodium 2-[5-(4-chlorophenyl)-pentyl]-oxirane-2-carboxylate (B 807–27) produces a dose-dependent hypoglycaemic effect when administered orally or intravenously to several fasted laboratory animals, i.e. rats (with and without adrenalectomy), guinea pigs, dogs, streptozotocin-treated diabetic pigs and db/db-mice. In addition, the substances have a more pronounced lowering effect on ketone bodies in the blood than any other known substance. The minimal dose for lowering blood glucose significantly in rats is 15 $\mu\text{mol/kg}$. The corresponding dose for a significant lowering of ketone bodies in the blood is < 2.4 $\mu\text{mol/kg}$. Hence, the substance B 807–27 is approximately five times more potent than tolbutamide and 30 times more potent than the biguanide buformin with respect to lowering blood glucose levels. B 807–27 differs from the sulphonylureas in that it fails to stimulate insulin secretion. In contrast to the biguanides, the substance decreases rather than increases blood lactate concentration and blocks both fatty acid oxidation and gluconeogenesis. The therapeutic usefulness of these compounds in diabetic man remains to be elucidated.

Key words: Hypoglycaemic agent, oxirane carboxylic acid, rat, adrenalectomised rat, guinea pig, dog, streptozotocin-diabetic pig, db/db-mice, plasma non-esterified fatty acid, blood ketone bodies, glucose, lactate.

An extrapancreatic effective therapeutic agent for the treatment of diabetes mellitus is not available apart from metformin. Evidence has accumulated re-

cently that several substituted or modified alcanoic acids are able to inhibit fatty acid oxidation and/or gluconeogenesis [1–7]. This inhibition, in accordance with Randle's 'glucose-fatty acid-cycle' [8], can cause a decrease in blood glucose concentration in vivo, especially in situations in which gluconeogenesis and fatty acid oxidation are very active, for example in diabetes or starvation. Chase and Tubbs [7] were the first to show that the co-enzyme A-derivative of the α -substituted alcanoic acid, 2-bromopalmitic acid, inhibits fatty acid oxidation. An even more potent compound, methyl 2-tetradecylglycidate (McN 3716) was reported in 1978 by Tutwiler et al. [2, 9]. Moreover, ω -phenylvaleric acid, described by DeGaldeano et al. in 1973, inhibits gluconeogenesis in the isolated perfused rat liver [10]. In the present study, we describe the pharmacological profile of the most effective compound of a new class of hypoglycaemic substances [11].

Materials and Methods

Animals

Male rats of the Sprague-Dawley strain (Ivanovas 50, Kisslegg, FRG), weighing 160–200 g, were used. Since the hypoglycaemic action of any effective compound is increased in adrenal insufficiency, some rats were adrenalectomized to augment the anticipated hypoglycaemic effect of B 807–27. Adrenalectomy was performed on 36 rats by a surgical procedure [12] 5 days before the experiment. After the operation, the animals received 0.9% NaCl-solution ad libitum instead of water. Male guinea pigs of the white inbred SPF-strain (Süddeutsche Versuchstierzucht, Tuttlingen, FRG), weighing 250–300 g, were used. Diabetic mice of the strain C57BL/KsJ-db/db were bred from 50 breeding pairs purchased from the Jackson Laboratory, Bar Harbor, Maine, USA. The animals were kept in groups of four per standard cage in a room with fixed light/dark rhythm (12/12 h), 55%–60% relative air humidity and temperature 20°–22°C. The animals received, unless otherwise stated, a standard pellet diet (rats, mice: Altromin 1320; guinea pigs: Altromin

3020) purchased from Altromin, Lage, FRG. Water was available ad libitum. Four male healthy, non-diabetic beagle dogs, weighing 15–20 kg, were used for acute studies. The dogs were maintained on a meat diet (Chappi from Effem, Verden, FRG). During the experiment, the dogs were kept isolated in metabolic cages with water ad libitum. Two streptozotocin-diabetic Hanford pigs (90 and 25 kg) were tested in cooperation with Dr. M. Marshall, Munich University. The diabetic state was induced by injection of streptozotocin [13].

Drugs

The synthesis of sodium 2-[5-(4-chlorophenyl)-pentyl]-oxirane-2-carboxylate (B 807–27 mol. wt. 290.7) and the ethyl ester (B 807–06 mol. wt. 296.8) has been described elsewhere [11].

Phenformin (2-phenylethyl-biguanide hydrochloride) was obtained from Dr. Brunnengräber, Chemische Fabrik, Lübeck, FRG. Buformin (1-butylbiguanide hydrochloride, Batch No. 2036) was received from Società Italiana Medicinali Scandicci, Incisa Valdarno, Italy and 2-bromo-hexadecanoic acid from Fluka, Chemische Fabrik, Buchs, Switzerland. Enzymes and pyruvate were supplied by Boehringer, Mannheim, FRG. All other chemicals were reagent grade from Merck, Darmstadt or from Serva, Heidelberg, FRG. Nembutal (sodium pentobarbital), ad usum veterinarium, Abbott, Bad Segeberg, FRG, was used for anaesthesia in rats.

Analytical Procedures

Blood glucose (hexokinase-method) and lactate were measured according to the method of Richterich [14]. Plasma non-esterified fatty acids (NEFA) were analysed by the method of Duncombe [15], plasma triglycerides and cholesterol were analysed using kits from Boehringer, Mannheim, FRG. Ketone bodies (acetoacetate and 3-hydroxybutyrate) were determined in the neutralized (K_3PO_4) supernatants after deproteinization of blood samples with $HClO_4$ (1 mol/l) by a modified method according to Mellanby and Williamson [16]. Plasma immunoreactive insulin (IRI) was estimated by a modified coated charcoal immunoassay according to Herbert et al. [17] using ^{125}I -insulin and anti-serum from Becton Dickinson, Heidelberg, FRG. For calibration, rat insulin (Novo Research, Denmark) was used.

Methods

For the studies in rats, guinea pigs and mice, the ester (B 807–06) was administered as a neutral emulsion with Cremophor EL (BASF, Ludwigshafen, FRG) (ratio 1 : 1) and water and the Na-salt (B 807–27) as an aqueous solution. The volume used was 10 ml/kg (oral) and 1 ml/kg for IV administration (vena femoralis) at a dose range of 2.5–300 μ mol/kg body weight. The animals were fasted 16 h (rats and mice) and 48 h (guinea pigs), or were allowed access to food as indicated and water ad libitum. Dogs received the substance in hard gelatine capsules after a 24 h-fast. In streptozotocin-diabetic pigs, the substance was administered via an indwelling

catheter implanted in the vena cava superior. Details of the animals' maintenance, feeding, and the surgical procedure have been described previously [18]. Rats, guinea pigs, and mice were bled by puncturing the retro-orbital plexus under light ether anaesthesia (20–200 μ l samples). Dog blood samples were taken from the vena femoralis; pig blood samples were withdrawn via the implanted catheter (both without anaesthesia).

Oral Glucose Loading Test in db-Mice

Groups of 10 db-mice of both sexes were treated with placebo or B 807–27 (190 μ mol/kg, per os) once a day (10.00 h) for 4 days. On day 3, food was withdrawn in the afternoon. On day 4, 1 h after dosing, 5.6 mmol/l glucose/kg body weight was given orally as a 10% solution. Two blood samples were taken retro-orbitally before the glucose load and further samples were collected at 15, 30, 60, 90, and 120 min thereafter. Baseline mean was subtracted from glucose values, and the area under the 'excess curve' was calculated.

Statistical Methods

Intergroup comparison of changes due to treatment was performed by means of an analysis of variance (Anova) and subsequent Scheffé-contrasts on log-transformed intra-individual ratios, treatment/pretreatment. Dixon's method [19] was applied for elimination of possible outliers. An effective dose ED_{50} (25%) $\pm$ SEM was calculated from dose-response relationships by linear or log-linear regression techniques. ED_{50} (25%) means that 50% of the animals show at least 25% decrease of blood glucose level compared to the controls. For all other statistical comparisons, Student's t-test was used ($p < 0.05, 0.01, 0.001$; two-tailed).

Results

Non-diabetic Rats

Both sodium salt B 807–27 and ester B 807–06 produced a dose-dependent decrease of blood glucose in non-diabetic, fasted rats within 2 h after oral administration. In comparison to the control rats, the decrease was significant at a dose of 15 μ mol/kg ($p < 0.001$). At a high dose of B 807–27 (300 μ mol/kg), blood glucose decreased by 50% and hypoglycaemia persisted for > 12 h, returning to normal after 24 h (Fig. 1 a). At the same concentration, the ester B 807–06 led to a blood glucose decrease of 58%. The effective doses ED_{50} (25%) $\pm$ SEM were 0.03 ± 0.01 and 0.02 ± 0.02 mmol/kg for B 807–27 and B 807–06, respectively. After IV administration, the hypoglycaemic effect was slightly greater than after oral administration, although this difference was not statistically significant (Table 1). Blood lactate levels were slightly but not significantly decreased after both, oral and IV administration (Table 1). B 807–27 given orally to fed rats was without effect at doses ≤ 150 μ mol/kg, although weak activity was detected at higher doses (Table 1).

B 807–27 as well as the corresponding ester B 807–06 (data not shown) caused lowering of ketone

Fig. 1. a Dose-dependent decrease of blood glucose concentration in fasted rats after oral administration of B 807-27. Doses ($\mu\text{mol/kg}$): Control ($\square$), 8 ($\bullet$), 15 ($\blacktriangle$), 75 ($\blacksquare$), and 300 ($\blacklozenge$). Results are expressed as mean $\pm$ SEM of 12 rats per dose group. **b** Dose-dependent decrease of blood glucose concentration in 48 h fasted guinea pigs after oral administration of B 807-27. Doses ($\mu\text{mol/kg}$): Control ($\square$), 7 ($\bullet$), 14 ($\blacktriangle$), 28 ($\blacksquare$), and 56 ($\blacklozenge$). Results are expressed as mean $\pm$ SEM of 12 animals per dose group

Fig. 2. Dose-dependent change of blood 3-hydroxybutyrate (3-OHB) + acetoacetate (AcAc) concentration in fasted rats after oral administration of B 807-27. Doses ($\mu\text{mol/kg}$): Control ($\square$), 8 ($\bullet$), 25 ($\blacktriangle$), 75 ($\blacksquare$), and 150 ($\blacklozenge$). Results are expressed as mean $\pm$ SEM of 12 rats per dose group

Fig. 3. Dose-dependent increase of plasma non-esterified fatty acid (NEFA) concentration in fasted rats after oral administration of B 807-27. Doses ($\mu\text{mol/kg}$): Control ($\square$), 8 ($\bullet$), 25 ($\blacktriangle$), 75 ($\blacksquare$), and 150 ($\blacklozenge$). Results are expressed as mean $\pm$ SEM of 12 rats per dose group

levels in fasted rats (Fig. 2). The decrease occurred between 2–12 h in a dose-dependent manner. In contrast, the lowering effect in fed rats was only moderate (Table 1). 3-Hydroxybutyrate (3-OHB) concentrations fell more markedly than those of acetoacetate. A dose of B 807-27 ($2.5 \mu\text{mol/kg}$) led to a 30%–50% decrease of acetoacetate (AcAc) levels and a 60%–75% decrease of 3-OHB levels 2–6 h after dosing ($p < 0.001$). The 3-OHB/AcAc ratio dropped from 2.4 to 0.8 at 4 h but was normal at 12 h after dosing with B 807-27 ($8 \mu\text{mol/kg}$). Higher doses (75, 150 $\mu\text{mol/kg}$) gave no significant change in 3-OHB/AcAc ratios.

Plasma NEFA were elevated in a dose-dependent manner (Fig. 3). At a low dose of B 807-27 ($8 \mu\text{mol/kg}$), NEFA levels increased approximately 300% at 2–4 h after dosing ($p < 0.001$), returning to normal 20 h later. There was no difference between per os and IV administration and no increase in NEFA was observed in fed rats (Table 1). Plasma triglyceride concentration increased up to 290% at 3 h after a dose of B 807-27 ($55 \mu\text{mol/kg}$) (Table 1). In parallel with NEFA, the triglyceride levels fell to normal 24 h later. No change was seen in the cholesterol concentration up to 5 h after administration. Twenty-four h after dosing with B 807-27 ($55 \mu\text{mol/kg}$), a decrease of 43% was estimated ($p < 0.001$). In fed rats, there were no changes in triglyceride and cholesterol levels (Table 1).

Adrenalectomized Rats

The hypoglycaemic reaction was more pronounced in adrenalectomized rats than in normal rats. A dose of B 807-27 ($25 \mu\text{mol/kg}$) produced a 34% decrease of blood glucose in adrenalectomized rats. B 807-27 ($150 \mu\text{mol/kg}$) led to death in all animals in this group

Table 1. Effect of the compound B 807–27 4 h after oral or intravenous administration of 75 $\mu\text{mol/kg}$ on some biochemical parameters in blood of fasted and fed rats compared with controls

Parameter		Fasted rats		Fed rats	
		per os	intravenous	per os	intravenous
Blood glucose (mmol/l)	Control	4.70 $\pm$ 0.08 (12)	4.76 $\pm$ 0.13 (12)	6.78 $\pm$ 0.19 (12)	6.22 $\pm$ 0.12 (10)
	Treated	3.08 $\pm$ 0.09 ^a (12)	2.92 $\pm$ 0.13 ^a (12)	7.51 $\pm$ 0.13 (12)	5.86 $\pm$ 0.07 (9)
Blood lactate (mmol/l)	Control	1.06 $\pm$ 0.08 (12)	0.98 $\pm$ 0.09 (12)	1.89 $\pm$ 0.12 (12)	1.41 $\pm$ 0.08 (10)
	Treated	0.79 $\pm$ 0.05 (12)	0.64 $\pm$ 0.03 (11)	1.97 $\pm$ 0.10 (10)	1.53 $\pm$ 0.18 (10)
Plasma NEFA (mmol/l)	Control	1.06 $\pm$ 0.08 (12)	1.18 $\pm$ 0.09 (12)	0.38 $\pm$ 0.05 (12)	–
	Treated	3.02 $\pm$ 0.06 ^a (12)	4.13 $\pm$ 0.17 ^a (12)	0.44 $\pm$ 0.02 (12)	–
Blood acetoacetate (mmol/l)	Control	0.29 $\pm$ 0.02 (11)	0.30 $\pm$ 0.02 (12)	–	–
	Treated	0.07 $\pm$ 0.01 ^a (11)	0.11 $\pm$ 0.01 ^a (11)	–	–
Blood 3-OHB (mmol/l)	Control	1.39 $\pm$ 0.07 (12)	1.18 $\pm$ 0.08 (12)	0.31 $\pm$ 0.09	–
	Treated	0.23 $\pm$ 0.02 ^a (12)	0.23 $\pm$ 0.02 ^a (12)	0.23 $\pm$ 0.05	–
Plasma triglycerides (mmol/l)	Control	0.47 $\pm$ 0.05 (10)	–	0.80 $\pm$ 0.04 (10)	–
	Treated	1.35 $\pm$ 0.09 ^{a, b} (10)	–	0.73 $\pm$ 0.07 ^b (10)	–
Plasma cholesterol (mmol/l)	Control	1.57 $\pm$ 0.10 (10)	–	1.93 $\pm$ 0.07 (10)	–
	Treated	1.79 $\pm$ 0.10 ^b (10)	–	1.91 $\pm$ 0.04 ^b (9)	–

Values represent mean $\pm$ SEM with the corresponding number of animals in parentheses. Level of significance: ^a $p < 0.001$; ^b3-h values at dose 0.055 mmol/kg

due to hypoglycaemic coma. Lactate levels were decreased (Table 2), but plasma NEFA levels increased three- to fivefold at 2–4 h after administration. As in normal rats, the ketone bodies decreased. The ratio 3-OHB/AcAc fell from 2.3 to 0.3 at 4 h after administration of B 807–27 (25 $\mu\text{mol/kg}$) (Table 2).

Non-diabetic Guinea Pigs

The hypoglycaemic effect in guinea pigs after oral administration of B 807–27 (7–56 $\mu\text{mol/kg}$) is shown in Figure 1b. The effect was of the same order of magnitude (ED_{50} (25%) $\pm$ SEM: 0.03 $\pm$ 0.01 mmol/kg), but the kinetics of blood glucose change was different compared with rats. The maximum decrease of blood glucose in 48-h fasted animals was seen between 2–3 h after administration. The duration of effect was short compared with rats, reaching baseline levels 5 h after dosing.

Dogs, Streptozotocin-Diabetic Pigs, and Genetic-Diabetic Mice (db/db)

To test the hypoglycaemic action of B 807–27 in non-rodents or diabetic animals, acute experiments were carried out with four beagle dogs, two streptozotocin-diabetic pigs (Hanford miniature pigs), and genetic-diabetic mice (db/db). The results are shown in Table 3.

In dogs, B 807–27 (100 $\mu\text{mol/kg}$, per os) caused a rapid lowering of blood glucose levels in the first 4 h after dosage. In streptozotocin-diabetic pigs and genetic-diabetic mice, the glucose-lowering effect increased over a 6 h period. Because large inter-individual variations of blood glucose concentration were seen in db/db-mice following a 16 h fasting period, we tried to obtain more exact data on the anti-diabetic effect of the compound by using an oral glucose load following repeated substance administration.

Table 2. Effect of the compound B 807-27 after oral administration of 25 $\mu\text{mol/kg}$ on some biochemical parameters in blood of adrenalectomized (AE) rats compared with controls

Parameter		Hours after administration (per os)				
		0	2	4	8	12
Blood glucose (mmol/l)	Control	3.36 $\pm$ 0.08 (10)	3.29 $\pm$ 0.07 (9)	3.58 $\pm$ 0.07 (9)	3.27 $\pm$ 0.16 (9)	3.08 $\pm$ 0.19 (9)
	AE-rats	3.35 $\pm$ 0.08 (10)	2.30 $\pm$ 0.13 ^c (10)	2.52 $\pm$ 0.17 ^c (10)	2.40 $\pm$ 0.16 ^c (8)	2.65 $\pm$ 0.16 ^a (7)
Blood lactate (mmol/l)	Control	0.82 $\pm$ 0.07 (10)	0.68 $\pm$ 0.07 (9)	0.99 $\pm$ 0.19 (10)	0.81 $\pm$ 0.06 (9)	1.24 $\pm$ 0.22 (9)
	AE-rats	0.78 $\pm$ 0.06 (10)	0.41 $\pm$ 0.04 ^a (9)	0.52 $\pm$ 0.02 ^a (9)	0.62 $\pm$ 0.05 (8)	0.72 $\pm$ 0.06 (7)
Plasma NEFA (mmol/l)	Control	0.50 $\pm$ 0.03 (10)	0.34 $\pm$ 0.04 (10)	0.41 $\pm$ 0.05 (10)	0.55 $\pm$ 0.06 (9)	0.27 $\pm$ 0.04 (9)
	AE-rats	0.54 $\pm$ 0.04 (10)	1.34 $\pm$ 0.07 ^c (10)	1.27 $\pm$ 0.10 ^c (10)	1.02 $\pm$ 0.04 ^a (6)	0.86 $\pm$ 0.07 ^c (7)
Blood 3-OHB + acetoacetate (AcAc) (mmol/l)	Control	1.18 $\pm$ 0.09 (10)	1.24 $\pm$ 0.06 (9)	1.32 $\pm$ 0.11 (9)	1.30 $\pm$ 0.09 (9)	1.66 $\pm$ 0.13 (9)
	AE-rats	0.98 $\pm$ 0.07 (10)	0.42 $\pm$ 0.02 ^c (8)	0.30 $\pm$ 0.06 ^c (10)	0.27 $\pm$ 0.07 ^c (8)	0.49 $\pm$ 0.09 ^c (7)
3-OHB/AcAc-ratio	Control	2.26 $\pm$ 0.14 (10)	2.33 $\pm$ 0.17 (9)	2.82 $\pm$ 0.45 (10)	1.91 $\pm$ 0.21 (9)	2.79 $\pm$ 0.41 (9)
	AE-rats	2.31 $\pm$ 0.16 (10)	0.70 $\pm$ 0.11 ^c (8)	0.31 $\pm$ 0.09 ^c (6)	0.34 $\pm$ 0.13 ^c (4)	0.38 $\pm$ 0.10 ^c (6)

Values represent mean $\pm$ SEM with the corresponding number of animals in parentheses. Level of significance: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$

Fig. 4. Oral glucose loading test in diabetic mice (*db/db*) after 4 days' administration of B 807-27 (190 $\mu\text{mol/kg}$) per os. The last administration was 1 h before the glucose load (5.6 mmol/kg). Results are expressed as mean $\pm$ SEM of 10 mice. The 'excess area' under the curve was calculated by integration of the concentration curve above the basal level at 0 min. 'Excess area' under the curve values are: Control group ($\square$), 1341 $\pm$ 71, treated group ($\bullet$), 663 $\pm$ 34 ($p < 0.001$). Results are expressed as mean $\pm$ SEM of 10 mice per group

Oral Glucose Loading Test in Diabetic Mice (*db/db*)

Genetic-diabetic mice (*db/db*) develop between 10–20 weeks of life span a diabetic syndrome with hyperglycaemia, obesity, hyperinsulinaemia and im-

Fig. 5. Dose-response relationships of B 807-27 ($\square$), tolbutamide ($\bullet$), and buformin ($\blacktriangle$) in fasted rats 3–5 h after oral administration. The percentage of animals with at least 25% decrease in blood glucose is shown in relation to the dose. Results are expressed as mean of 12 rats per dose group. Regression lines follow the equation: $y = a \times \ln(x) + b$. Regression coefficients (r) are: 0.993 ($\square$), 0.963 ($\bullet$) and 0.949 ($\blacktriangle$)

paired glucose tolerance, comparable to insulin-dependent diabetes in man.

B 807-27 (190 $\mu\text{mol/kg}$) per os per day for 4 days (the last dose being given 1 h before the glucose load, 5.6 mmol/kg, per os) produced not only a strong hy-

Table 3. Percentage decrease of blood glucose in several species after administration of B 807–27 compared with baseline (dog, pig) or control values (rat, guinea pig, mouse), respectively

Animal	Treatment		Percentage glucose decrease at increasing time after administration		
	Dose ($\mu\text{mol/kg}$)	Form of administration	2 h	4 h	6 h
Dog	100	per os	37 ± 5 (4)	25 ± 9 (4)	9 ± 5 (4)
Streptozotocin-diabetic pig	40	IV	20 (1)	33 (1)	35 (1)
	10	IV	12 (1)	17 (1)	–
Diabetic mouse (<i>db/db</i>)	70	per os	25 ± 9 (10)	22 ± 9 (10)	39 ± 9 (10)
Rat	30	per os	22 ± 1 (24)	25 ± 1 (24)	22 ± 1 (24)
Guinea pig	30	per os	30 ± 4 (12)	25 ± 4 (12)	10 ± 4 (12)

Results are expressed as mean $\pm$ SEM with the corresponding number of animals in parentheses

Table 4. Some hypoglycaemic compounds compared with B 807–27. Comparison of effects and proposed mechanisms of action

Compound	Hypoglycaemic effect in fasted rats ED_{50} (25%) mmol/kg ^a	Proposed mechanism of action	Reference
Tolbutamide (sulphonylurea)	0.14 ± 0.02	Stimulation of insulin secretion	For a review Bänder [20]
Buformin (biguanide)	1.00 ± 0.40	Inhibition of gluconeogenesis, intestinal glucose absorption and non-specific substrate oxidation in mitochondria	Beckmann [21] Schäfer [22]
2-Bromopalmitic acid	0.55 ± 0.23	Inhibition of carnitine palmitoyl-transferase (EC 2.3.1.21) and long-chain fatty acid oxidation	Chase and Tubbs [7]
ω -Phenylvaleric acid	> 1.7	Inhibition of gluconeogenesis	DeGaldeano et al. [10]
Methyl 2-tetradecylglycidate (McN-3716)	about 0.13	Inhibition of mitochondrial carnitine palmitoyltransferase I (EC 2.3.1.21) and long-chain fatty acid oxidation	Tutwiler and Ryzlak [25]
B 807–27 Sodium 2-[5-(4-chlorophenyl)-pentyl]-oxirane-2-carboxylate	0.03 ± 0.01	Inhibition of carnitine palmitoyl-transferase I (EC 2.3.1.21) with consequent inhibition of long-chain fatty acid oxidation and gluconeogenesis	Wolf et al. [31]

^a Values express ED_{50} (25%) $\pm$ SEM, calculated from dose-response relationships with 12 rats per dose group

poglycaemic effect on the baseline glucose but also lowered the 'excess area' under the glucose concentration curve (Fig. 4). The glucose baseline decreased from 7.87 ± 2.97 to 3.26 ± 1.78 mmol/l (mean $\pm$ SEM, $n = 10$, $p < 0.001$) after a 16 h overnight fast. The 'excess area' under the curve was significantly reduced to about half of the control value ($p < 0.001$). No change was seen in the pattern of the glucose concentration curve (Fig. 4).

Discussion

In this study, a selected potent agent of a new class of hypoglycaemic compounds [11] is shown to produce a dose-dependent decrease of glucose and ketone bodies in several normal and diabetic animals. Since

B 807–27 has the same potency as its ester B 807–06, and no significant difference exists between per os and IV administration, it seems likely that the anion of B 807–27 is the active compound reaching the site of action in the target tissues.

In comparison to some more recently described inhibitors of long-chain fatty acid oxidation, B 807–27 is different in several respects. Despite possible similarity in the mechanism of action, B 807–27 has a strict dose-dependent lowering effect on blood glucose ten times that of α -bromopalmitate (Table 4) and also appears more potent than methyl 2-tetradecylglycidate (McN-3716) in non-diabetic fasted rats [2]. In contrast to McN-3716 [9], B 807–27 has a prompt but small hypoglycaemic effect in dogs. In long-term experiments, B 807–06 is tolerated at doses ≤ 675 $\mu\text{mol/kg}$ in rats fed with normal rat chow,

without causing anorexia or weight loss. The compound lowers blood ketone bodies to a greater extent than any other known substance. The minimum dose (per os) for significant lowering of ketone bodies is $< 2.0 \mu\text{mol/kg}$.

Compound B 807-27 has no effect on plasma insulin concentration in fasting rats, or on insulin secretion in isolated perfused rat pancreas. Thus, although B 807-27 differs in this respect from the sulphonylureas, it does exert an insulin-like effect on glucose and ketone levels, but without an antilipolytic effect. In lowering the blood glucose level, the substance B 807-27 is about 30 times more effective than the biguanide buformin and about five times more potent than tolbutamide. As the dose-response curves are not parallel (Fig. 5), the above potencies are referenced to the ED_{50} (25%) $\pm$ SEM. These values are $0.03 \pm 0.01 \text{ mmol/kg}$ for B 807-27, $1.00 \pm 0.40 \text{ mmol/kg}$ for buformin, and $0.14 \pm 0.02 \text{ mmol/kg}$ for tolbutamide. By comparison, the ED_{50} (25%) for α -bromopalmitate is approximately 0.6 mmol/kg , whereas phenformin is completely inactive up to dose levels of 1.67 mmol/kg body weight.

In contrast to buformin, which produces a sustained and marked increase in blood lactate levels in rats even at doses that do not lower blood glucose, no increase in blood lactate concentration was seen in normal rats and guinea pigs with B 807-27 at equipotent doses and in adrenalectomized rats lactate concentrations actually decreased. Thus, the compound does not appear to inhibit the mitochondrial respiratory chain, as described for the biguanides [22]. This observation is supported by the lowering of 3-OHB/AcAc-ratios in blood after B 807-27 administration (Table 1), indicating a more oxidized state in mitochondria, although, concomitant to blood glucose decrease, the plasma NEFA levels increase. This may be caused by inhibition of carnitine palmitoyl transferase I (EC 2.3.1.21), a key enzyme of long-chain fatty acid oxidation [2, 7, 25, 26].

Hypoglycaemia can be counteracted by hormones such as catecholamines which stimulate lipolysis in adipose tissue. Apart from the inhibitory effect of B 807-27 on NEFA utilization, it is possible that catecholamines contribute to the three- to fivefold increase of NEFA in plasma (Fig. 3). In adrenalectomized rats, counteracting hormone levels are reduced, which might, therefore, account for the compound acting more strongly. The rats die in hypoglycaemic coma at a dose lowering blood glucose by 40% in normal rats. The increase of NEFA in plasma is of the same order of magnitude, but absolute levels are lower than in normal rats (Tables 1 and 2).

Elevation of plasma NEFA levels stimulate the production of secretory low density lipoproteins in

the liver [27]. We observed an increase of triglycerides in fasted rats parallel to the rise in NEFA levels. It is important for therapeutic use to emphasize that an increase of NEFA and triglycerides occur only in acute experiments with fasting animals but not in long-term experiments in the fed state (unpublished observations).

Two further findings support the assumption that inhibition of long-chain NEFA oxidation by B 807-27 can be used therapeutically in diabetes: Firstly, B 807-27 lowers elevated blood glucose levels in streptozotocin-diabetic pigs and secondly, the compound reduces glucose concentrations after a glucose load in genetic-diabetic mice (*db/db*) (Fig. 4). Considering that the liver represents the primary site of insulin action on carbohydrate metabolism following a glucose load – as shown by several groups [28–30] – and that diabetes is characterized by stimulated gluconeogenesis and fatty acid oxidation, the response to a glucose load in diabetic mice treated with B 807-27 may well be a consequence of inhibition of enhanced gluconeogenesis.

In conclusion, the new compound has insulin-like properties, i.e. it inhibits gluconeogenesis and ketogenesis by inhibition of long-chain fatty acid oxidation, and diverts NEFA to the re-esterification sequence in the liver. Investigations concerning the site of action and toxicological properties are in progress.

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