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Hypoglycaemic effect of metformin in genetically obese (*fa/fa*) rats results from an increased utilization of blood glucose by intestine

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The insulin-resistant obese *fa/fa* rat is a convenient model in which to study a potential effect of metformin, a biguanide used in the treatment of non-insulin-dependent diabetes, on insulin-mediated glucose utilization. Female *fa/fa* rats were given metformin orally for 8 days. Studies were performed on anaesthetized post-absorptive rats 5 h after the last dose of metformin. Glucose production and utilization were enhanced 1.5-fold in metformin-treated rats. The enhanced glucose production was almost entirely due to increased glucose recycling. The digestive tract was the only tissue responsible for the enhanced glucose utilization.

INTRODUCTION

Metformin is an antihyperglycaemic drug used in the treatment of non-insulin-dependent diabetes. The antihyperglycaemic action of metformin has been generally attributed to a combination of a decreased rate of intestinal absorption of carbohydrate, a decreased hepatic gluconeogenesis and an improvement of peripheral glucose utilization (Meyer *et al.*, 1967; Caspary & Creutzfeldt, 1971; Lorch, 1971; Frayn & Adnitt, 1972; Hermann, 1979; Nosadini *et al.*, 1987). It has been shown that *in vitro* metformin increased glucose uptake in skeletal muscles and white adipose tissue, two major insulin-dependent tissues (Gigolini *et al.*, 1984; Bailey & Puah, 1986). However, studies *in vivo* in man have suggested that metformin acts principally on splanchnic tissues (Jackson *et al.*, 1987).

Human type II non-insulin-dependent diabetics are hyperglycaemic. This hyperglycaemia is due to a combination of insulin-resistance and insulin deficiency (De Fronzo *et al.*, 1983). The obese (*fa/fa*) rat, like the human type II diabetic, is markedly insulin-resistant at both the hepatic and the peripheral level (Jeanrenaud *et al.*, 1985). The *fa/fa* rat in the post-absorptive state is normoglycaemic, but at the expense of a marked hyperinsulinaemia. After carbohydrate ingestion, the *fa/fa* rat is markedly glucose intolerant (Rohner-Jeanrenaud *et al.*, 1986).

The aim of the present work was to study the possible effect of metformin on insulin action *in vivo* in this markedly insulin-resistant animal.

MATERIALS AND METHODS

Animals

Obese female *fa/fa* rats were purchased from the Centre de Sélection et d'Elevage d'Animaux de Laboratoire (C.N.R.S., Orléans, La Souce, France). They were 9 weeks old at receipt and were fed *ad libitum* on a standard laboratory diet. They were housed in individual cages in animal quarters in which the temperature was maintained at 23 ± 1 °C and light was on from 07:00 to 19:00 h. They were treated daily, for 8 days, at 09:00 h with 350 mg of metformin (70 mg/ml)/kg body wt., orally, or with an equivalent volume of water. This dosage of metformin is high when compared with human studies, but is in the range of that allowing a decrease in the glycaemia of hyperglycaemic rodents when given orally (Lorch, 1971; Junien *et al.*, 1981; Kakemi *et al.*, 1983).

Surgery

Rats were deprived of food for 6 h before the experiments, since it has been shown that in these conditions the gut no longer delivers glucose into the circulation (Leturque *et al.*, 1981). Rats were anaesthetized with sodium pentobarbitone (50 mg/kg, intraperitoneally). The right carotid artery was catheterized for blood sampling, and a tracheotomy was performed to avoid respiratory difficulties during anaesthesia. Injections or perfusions were performed through saphenous veins. Body temperature was maintained at 37 °C with heating lamps and isotherm pads. A stabilization period of 30 min followed these surgical procedures, in order to reach a new steady state for blood glucose concentration and glucose turnover rate (Pénicaud *et al.*, 1987*a*).

Measurement of glucose turnover rate

A tracer dose of [3-^{3}H]glucose (40 μCi; Amersham) and [U-^{14}C]glucose (20 μCi) in 200 μl of 0.9% NaCl was injected as a bolus via the saphenous vein. Blood samples were drawn via the carotid artery at 1, 3, 5, 10, 20, 30, 45, 60 and 90 min after the injection. They were immediately deproteinized in $HClO_4$ (6%, v/v), neutralized and centrifuged (10000 *g* for 5 min). Glucose specific radioactivity was determined as follows: glucose concentration was measured in the supernatant with a glucose oxidase kit (Boehringer, Meylan, France). Another sample of the supernatant was evaporated to dryness to remove

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^3H_2O. The dry residue was redissolved in 200 μl of water and passed through ion-exchange resin columns (internal diameter 5 mm) containing 2.5 cm of Dowex AG1X8 (formate form) and 2.5 cm of Dowex AG50WX8 (H^+ form) to remove the ^{14}C-labelled metabolites; 1.3 ml of water was then passed through the column. A 1 ml sample of the eluate was taken and the radioactivity was measured after addition of 9 ml of scintillation fluid, by using a liquid-scintillation spectrometer with double channels and automatic correction for the quenching and spill-over of ^{14}C into the ^3H channel. At time 90 min, the blood glucose specific radioactivity for both tracers was < 5% of the 1 min value, thus allowing us to calculate glucose turnover by using the formula (dose of labelled glucose injected)/($_0\int^{60\ min}$ glucose specific radioactivity). Glucose recycling was taken as the difference between [3-^3H]glucose and [U-^{14}C]glucose turnover rates.

Measurement of glucose utilization index in individual tissues

A tracer dose of 2-deoxy[1-^3H]glucose (30 μCi, 20 Ci/mmol; C.E.A., Saclay, France) was injected in 100 μl of 0.9% NaCl as a bolus through a saphenous vein. Blood was sampled at 1, 3, 5, 10, 20, 30, 45 and 60 min after the injection via the arterial catheter for determination of 2-deoxy[1-^3H]glucose and blood glucose concentration. After the last blood sample, the rat was killed by cervical dislocation. Soleus and extensor digitorum longus muscles of each hind-leg and epitrochlearis of each foreleg, periovarian white adipose tissue, stomach, a portion of the jejunum and of the duodenum were immediately removed, and their 2-deoxy[1-^3H]glucose 6-phosphate contents were determined as described previously (Ferré *et al.*, 1985). The muscles studied are representative of a large range of fibre-type composition, since the composition in terms of slow-twitch oxidative, fast-twitch oxidative glycolytic and fast-twitch glycolytic fibres is respectively 84%, 16%, 0% for the soleus, 3%, 59%, 38% for the extensor digitorum longus, and 15%, 15%, 70% for the epitrochlearis (Ariano *et al.*, 1973; Nesher *et al.*, 1985). Glucose utilization in each tissue was calculated as described previously (Ferré *et al.*, 1985), except that the values were not corrected by the discrimination factor (lumped constant) for 2-deoxyglucose in glucose-metabolic pathways. Thus the results must be considered as an index of glucose utilization rather than an absolute value.

Hyperglycaemic glucose clamp

In a group of metformin-treated rats, glycaemia was increased to the level of controls by an hyperglycaemic glucose clamp. Briefly, glucose (10%, w/v) was infused through a saphenous vein, and glycaemia was measured every 5 min by using a glucose analyser (YSI 27; Yellow Springs Instruments Co., Yellow Springs, OH, U.S.A.); thereafter the rate of glucose infusion was adjusted accordingly. The hyperglycaemic glucose clamp was achieved when a steady state for glycaemia and glucose infusion rate was reached. This usually takes 30–40 min. 2-Deoxy[1-^3H]glucose was then injected and glucose utilization in tissues was determined as described above.

Analytical methods

Blood samples (50 μl) were deproteinized in 250 μl of $ZnSO_4$ (2.51%, w/v)/250 μl of $Ba(OH)_2$ (2.63%, w/v) and immediately centrifuged (10000 ***g*** for 5 min). Glucose concentration was measured on a sample of the supernatant by a glucose oxidase method (kit from Boehringer, Mannheim, Germany). Another sample was either directly counted for radioactivity in a liquid-scintillation spectrometer to determine 2-deoxy[1-^3H]glucose, or previously evaporated to dryness in order to remove the ^3H_2O and redissolved in 200 μl of water. At the end of each experiment a larger blood sample was taken. A sample was deproteinized in $HClO_4$ (6%, w/v) and centrifuged (10000 ***g***). The neutralized supernatant was used for the measurement of lactate and 3-hydroxybutyrate by specific enzymic methods (Williamson *et al.*, 1962; Passonneau, 1974). Another sample was centrifuged (10000 ***g***) at 4 °C, and the plasma was frozen for subsequent determination of pancreatic hormones. Plasma insulin and glucagon were determined by radioimmunoassay (kit from ORIS Industrie, Gif/Yvette, France, and kit no. 10904 from Serono Diagnostic S.A., Coinsins, Switzerland, respectively).

A lobe of liver was rapidly dissected and deep-frozen in liquid N_2 for the determination of glycogen content (Roehrig & Allred, 1974).

Statistics

Results are expressed as means ± S.E.M. and statistical significance of differences was assessed by Student's *t* test for unpaired data.

RESULTS

Body weight, substrates and hormones

Body weight was not affected by the short-term treatment with metformin: 311 ± 8 g ($n = 20$) for control and 307 ± 6 g ($n = 26$) for treated rats.

Metformin induced a 17% decrease in glycaemia and a significant fall in insulinaemia, but no change in plasma glucagon concentration (Table 1). Blood lactate was increased by metformin, whereas 3-hydroxybutyrate was not altered by the treatment (Table 1). There was a lower liver glycogen concentration in metformin-treated rats compared with controls (4.8 ± 0.8 versus 9.3 ± 2.3 mg/g wet wt. of liver; $P < 0.01$, $n = 6$).

Whole-body glucose kinetics

In order to analyse the reasons for the decreased glycaemia in metformin-treated rats, we measured whole-body glucose kinetics. There was a 30% increase in whole-body glucose turnover rate in rats treated with metformin (Table 1). This increase was mainly due to increased glucose recycling (Table 1). Since rats were in the post-absorptive state, it indicates that metformin induces an increase in liver glucose production and in glucose utilization by peripheral tissues.

Glucose utilization in individual tissues

Basal state. To determine which tissues could be involved in the increased glucose utilization, we have used the 2-deoxy[1-^3H]glucose method (Ferré *et al.*, 1985). Metformin treatment induced a 35% decrease in glucose utilization in skeletal muscles (Table 2). However, this decrease was significant only in the extensor digitorum longus and in the epitrochlearis. Glucose utilization in the periovarian white adipose tissue was not affected by the metformin treatment.

The only tissue studied that showed a large (39%)

Table 1. Circulating hormones and substrates and glucose kinetics in control, metformin-treated and metformin-treated hyperglycaemic rats

Results are means ± S.E.M. for 5–12 determinations: *, ** Difference statistically significant at respectively $P < 0.05$ and $P < 0.01$ compared with controls; †, †† difference statistically significant respectively at $P < 0.05$ and $P < 0.01$ compared with metformin-treated rats. ND, not determined. For further details see the text.

	Control	Metformin-treated	Metformin-treated + hyperglycaemic clamp
Blood glucose (mmol/l)	5.5±0.05	4.55±0.05**	5.88±0.15††
Blood lactate (mmol/l)	1.5±0.2	3.1±0.6*	6.8±0.8**,††
Blood β-hydroxybutyrate (mmol/l)	0.17±0.05	0.12±0.01	ND
Plasma insulin (μunits/ml)	423±33	295±37*	567±108†
Plasma glucagon (pg/ml)	404±14	426±14	ND
Glucose turnover rate (μmol/min per kg)	53±3	77±2**	89±5**,†
Glucose recycling rate (μmol/min per kg)	8.9±2	22±2**	ND

and significant increase in glucose utilization under metformin treatment was the jejunum.

Hyperglycaemic clamp. Since glucose utilization depends on both glycaemia and insulinaemia, and since these two parameters are decreased by the metformin treatment, this might induce an underestimation of the effects of metformin on glucose utilization by individual tissues. Thus glycaemia and insulinaemia were increased in metformin-treated rats to levels similar to those of control rats by a hyperglycaemic glucose clamp. Glucose turnover rates and glucose utilization by individual tissues were then measured. An exogenous glucose infusion of 8.0±0.6 mg/min per kg ($n = 6$) allowed equalization of glycaemia in metformin-treated and control rats (Table 1). Insulinaemia was slightly, but not significantly, higher during the hyperglycaemic clamp than in control rats.

At similar levels of glycaemia and insulinaemia, glucose turnover rate was 77% higher in metformin-treated rats than in controls, and was further increased as compared with metformin-treated rats (Table 1). At similar levels of glycaemia and insulinaemia, glucose utilization in skeletal muscles was identical in metformin-treated and control rats (Table 2), except in the epitrochlearis, where there was a small increase in glucose utilization with metformin treatment. Glucose utilization in white adipose tissue was not affected by metformin treatment (Table 2). The most dramatic effect of metformin was observed in the intestinal tissue, where glucose utilization reached more than 200% of control values (Table 2).

Table 2. Glucose utilization in individual tissues of control, metformin-treated and metformin-treated hyperglycaemic rats

Results are means ± S.E.M. for 5–9 determinations. Abbreviations: EDL, extensor digitorum longus; WAT, perioovarian white adipose tissue. Values are expressed as ng/min per mg wet wt.: *,** difference statistically significant at respectively $P < 0.05$ and $P < 0.01$ compared with controls; †, †† difference statistically significant respectively at $P < 0.05$ and $P < 0.01$ compared with metformin-treated rat. For other details see the text.

	Glucose utilization (ng/min per mg)		
Tissue	Control	Metformin-treated	Metformin-treated + hyperglycaemic clamp
Soleus	4.0±0.7	2.5±0.3	4.0±0.3
EDL	3.1±0.3	1.9±0.2*	2.5±0.2
Epitrochlearis	3.1±0.3	2.0±0.2*	4.2±0.5†
WAT	1.9±0.2	1.6±0.1	2.1±0.1
Stomach	3.9±0.7	3.6±0.7	8.3±0.3*,††
Duodenum	11.5±2.4	14.8±3.5	37.9±2.6**,††
Jejunum	14.3±0.9	20.0±2.3*	45.0±5.6**,††

DISCUSSION

In obese (*fa/fa*) rats, metformin lowers blood glucose and plasma insulin concentrations. This corroborates studies performed in *db/db* or *ob/ob* mice (Junien *et al.*, 1981; Lord *et al.*, 1983). This hypoglycaemic effect is due to an increase in glucose utilization, which is partially compensated by an increase in hepatic glucose production. Since at similar glycaemia plasma insulin levels are identical (Table 1), this suggests, as previously described (Hermann, 1979), that metformin does not act through an enhanced insulin secretion.

Glucose production

The increase in hepatic glucose production involves both an increased gluconeogenesis, as underlined by the increased glucose recycling (Table 1), and an increased glycogenolysis. This is at variance with studies showing a decreased glucose production under biguanide treatment (Meyer *et al.*, 1967; Ogata *et al.*, 1974; Hermann, 1979). However, it has been shown in the rat that the hypoglycaemic effect of another biguanide, phenformin, is independent of its hepatic effects (Polacek & Ouart, 1974) and that in rats after injury metformin does not inhibit hepatic gluconeogenesis from lactate (Frayn, 1976). In non-insulin-dependent diabetics, a modest decrease in basal hepatic production under metformin treatment has been shown (Jackson *et al.*, 1987; Nosadini

et al., 1987). The discrepancy between the effect of metformin on glucose production in man and rat could be linked to the fact that, in metformin-treated subjects in the basal state, blood lactate concentration was not increased in man (Jackson *et al.*, 1987), whereas it was markedly increased in the rat (Table 1), thus contributing to the enhanced glucose production through increased glucose recycling (Table 1).

Glucose utilization

Whole-body glucose utilization is increased by 30% in metformin-treated rats. In man, in non-insulin-dependent diabetics in the basal state, glucose utilization is not increased by metformin (Gigolini *et al.*, 1984; Jackson *et al.*, 1987). However, if the lower glycaemia in the metformin-treated subjects is taken into account, a 30–40% increase in glucose clearance rate is apparent.

Metformin has been shown to improve insulin-induced glucose utilization in non-insulin-dependent (Prager *et al.*, 1986; Jackson *et al.*, 1987) as well as in insulin-dependent (Pagano *et al.*, 1983; Gin *et al.*, 1985) diabetics.

In the present study, we could not detect changes in glucose utilization in insulin-dependent tissues. In skeletal muscles glucose utilization was even decreased by metformin when glycaemia was low, and returned to normal values when blood glucose and insulinaemia were raised to the values observed in control rats. Thus the marked insulin-resistance observed in skeletal muscles and white adipose tissue of obese (*fa/fa*) rats (Pénicaud *et al.*, 1987*b*) is not alleviated by a short-term metformin treatment. These results are in agreement with those of Jackson *et al.* (1987), who found no increase in muscle glucose uptake in man. Nevertheless, an improvement by metformin of tissue response to insulin in a rat model with a milder insulin resistance or after a longer metformin treatment cannot be excluded.

The striking result of the present study is that the increased glucose utilization takes place mainly in non-insulin-dependent tissues, stomach and small intestine.

It is well known that the digestive tract is one of the tissues where biguanides are the most concentrated and represents a preferential site for metformin effects (Wick *et al.*, 1960; Ghareb *et al.*, 1969; Kruger *et al.*, 1970; Caspary & Creutzfeldt, 1971; Molloy *et al.*, 1980).

In the present study, we report a marked effect *in vivo* of metformin on the utilization of blood glucose by the stomach and intestine. This is not contradictory with studies showing a decreased exogenous-glucose absorption by the intestine (Ghareb *et al.*, 1969; Kruger *et al.*, 1970; Caspary & Creutzfeldt, 1971), since two different glucose transport systems are involved for the entry of glucose into the intestinal epithelium from the mucosal and from the serosal sides (Kimmich & Randles, 1981). The mucosal glucose carrier system depends on the Na^{2+} electrochemical gradient and is energy-dependent. In contrast, the serosal glucose transport is a facilitated-diffusion system which is not energy-dependent.

Thus similarities in terms of mode of action of metformin between the study of Jackson *et al.* (1987) in man and the present study in rats are striking: metformin does not act through an increased insulin secretion, but increases glucose clearance. This is accompanied by an increased lactataemia, especially at high glycaemia; muscles (the main mass of insulin-sensitive tissues) are not implicated in the increased glucose clearance. Jackson *et al.* (1987) postulated an effect on splanchnic glucose utilization, especially by the liver. In our study, it is unlikely that the liver contributes to this increased glucose utilization, since hepatic glucose production was increased by the treatment. In contrast, it is clear that the intestine is a major target for biguanide action on glucose utilization. The only noticeable difference between their study and ours is the effect of the drug on glucose production, slightly decreased in their study (18%), but increased (40%) in our study. It must be pointed out, however, in addition to the above-mentioned difference in blood lactate concentration, that diabetic humans in the basal state are probably overproducing glucose (De Fronzo *et al.*, 1982), whereas obese (*fa/fa*) rats are not (Terretaz *et al.*, 1986).

In conclusion, hypoglycaemia induced by metformin was due to an increase in glucose utilization localized in the digestive tract. This induces, in turn, a decreased insulinaemia, an increased glucose production and a decreased liver glycogen concentration. This leads to a new steady state at a lower glycaemia.

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