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Importance of the intestine as a site of metformin-stimulated glucose utilization

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1 The intestine has been implicated as a site of increased glucose utilization by the antihyperglycaemic drug, metformin. This study makes a quantitative assessment of this effect.

2 Glucose utilization by the intestine and hind limb region was determined by arterial-venous glucose difference adjusted for blood flow rate in fasted rats receiving a hyperglycaemic hyperinsulinaemic infusion.

3 Intrajejunal administration of metformin, 250 mg kg^{-1}, increased glucose disposal during the infusion procedure, associated with increased glucose utilization in the intestine by 69% and in the hind limb region by 40%.

4 Metformin, 250 mg kg^{-1}, increased glucose disappearance during an intravenous glucose tolerance test. This was accompanied by increased uptake of tritiated 2-deoxy-D-glucose into the intestinal mucosa to a greater extent than into skeletal muscles (per unit wet weight of tissue).

5 The results demonstrate that the intestinal mucosa is a quantitatively important site of increased glucose utilization during the blood glucose-lowering effect of metformin.

Keywords: Metformin; glucose utilization; intestine; hind limbs

Introduction

The antihyperglycaemic agent, metformin (dimethylbiguanide), increases glucose utilization, reduces hepatic glucose production and reduces the rate of intestinal glucose absorption (Bailey, 1992; 1993). The blood glucose-lowering effect requires the presence of at least some insulin, but metformin does not increase insulin concentrations (Bailey & Mynett, 1994). Metformin augments insulin-stimulated glucose uptake and metabolism by skeletal muscle and fat, and increases the suppression of hepatic gluconeogenesis by insulin (Bailey, 1992; 1993).

Recent studies have shown that metformin increases glucose utilization by the intestine (Penicaud *et al.*, 1989; Bailey *et al.*, 1992). This occurs in the fasted state as well as in the fed state, indicating that the drug can increase the intestinal extraction of glucose from the vascular compartment. The intestine requires the presence of insulin to undertake normal glucose metabolism, but it does not show an insulin concentration-related utilization of glucose as seen with muscle and fat (Kellett *et al.*, 1984). Moreover, the intestinal wall accumulates much higher concentrations of metformin than other tissues (Wilcock & Bailey, 1994), raising the possibility that the drug may have a different mode of action in the intestine than in other tissues.

The present study investigates the contribution of the intestine to the overall effect of metformin on glucose uptake from the circulation. The study examines whether metformin alters blood flow through the intestine, and compares glucose utilization by the intestine and hind limb region after i.v. glucose administration. The uptake of glucose by different tissues has also been assessed using the non-metabolized glucose analogue, 2-deoxy-D-glucose.

Methods

Animals

Adult male Wistar rats weighing 150–200 g were maintained as previously described (Bailey *et al.*, 1992). Anaesthesia was induced with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and maintained with further doses of 15 mg kg^{-1} h^{-1}. Rectal temperature was held at 34–36°C. A cannula was introduced into the right internal jugular vein for i.v. administration of test substances.

Blood flow

Rats were fasted for 24 h, anaesthetized, and the abdomen was opened. Transonic ultrasound flow sensors (2 mm) were coupled around the hepatic portal vein (HPV) and lower abdominal vena cava (IVC) using acoustic lubricating jelly. The sensors were connected to a T106 transonic volume flow meter (Transonic System Inc, Ithaca, New York, U.S.A.) supplied by Linton Instrumentation, Diss, Norfolk. Blood flow was monitored continuously (Drost *et al.*, 1984) and recorded on a flat-bed chart recorder.

Hyperglycaemic hyperinsulinaemic infusion

Twenty-four hours fasted, anaesthetized rats received an i.v. infusion of the following via the jugular cannula: adrenaline bitartrate (0.08 μg kg^{-1} min^{-1}), propranolol hydrochloride (1.7 μg kg^{-1} min^{-1}), glucose (16 mg kg^{-1} min^{-1}) and bovine insulin (3 miu kg^{-1} min^{-1}) as described by Reaven *et al.* (1983). This produced a steady hyperglycaemia (about 11 mmol l^{-1}) and hyperinsulinaemia (about 5 ng ml^{-1}) by 60–90 min. This steady state was maintained in control animals throughout the remainder of the experiment (until 180 min). The effect of metformin was determined by administration of metformin hydrochloride (250 mg kg^{-1}) into the proximal jejunum at 90 min. Control animals received an equivalent volume of PBS, 10 ml kg^{-1}. Blood samples were taken from the tail tip at 30 min intervals throughout the study for determination of plasma glucose and insulin concentrations.

At the end of the infusion period (180 min), simultaneous blood samples were taken for plasma glucose determination from the lower abdominal aorta, lower abdominal vena cava, and hepatic portal vein.

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Tissue uptake of 2-deoxy-D-glucose

Glucose uptake into different tissues was measured using 2-deoxy-D-[1-^{3}H]-glucose administered during an intravenous glucose tolerance test (IVGTT). Twenty-four hour fasted, anaesthetized rats received either metformin hydrochloride (250 mg kg^{-1}) or an equivalent volume of PBS (10 ml kg^{-1}) delivered into the proximal jejunum at −60 min. At 0 min the rats received an i.v. glucose bolus (0.5 g kg^{-1}), and at 5 min an i.v. injection of 2-deoxy-D-[1-^{3}H]-glucose (5 μCi per 100 g body weight) and [U-^{14}C]-sucrose (1.67 μCi per 100 g body weight). Blood samples for plasma glucose determination were taken from the tail tip at −60, 0, 5 and 30 min. The rate of plasma glucose disappearance (% min^{-1}) was determined ($69.3/t_{\frac{1}{2}}$) between 5 and 30 min. At 30 min a venous blood sample and the following tissues were quickly removed: quadriceps femoris, soleus, transverse abdominal muscle, diaphragm, heart ventricular apex, epididymal white fat, antral region of stomach, mid-jejunum, mid-small intestine (halfway between ligament of Treitz and ileo-caecal valve), mid-ileum, descending colon, and cerebral hemisphere of brain. Samples of intestinal mucosa were carefully separated by scraping with a microscope slide. Pieces of tissue weighing about 50 mg and 50 μl of plasma were digested in 0.5 ml 1 mmol l^{-1} NaOH at 80°C, and 5 ml Hisafe II scintillant was added for counting on a Packard 1800 TR liquid scintillation analyser. The tissue accummulation of 2-deoxy[1-^{3}H]-glucose-6-phosphate was determined as d.p.m. mg^{-1} wet weight of tissue corrected for the sucrose space.

Glucose and insulin assays

Plasma glucose was measured by an automated glucose oxidase procedure (Stevens, 1971) and plasma insulin was determined by radioimmunoassay using a polyethylene glycol separation method (Desbuquois & Aurbach, 1971).

Chemicals

All chemicals were obtained from Sigma Chemical Company, Poole or BDH, Poole except: PBS tablets from Unipath, Basingstoke; metformin hydrochloride from Lipha Pharmaceuticals, West Drayton; sodium pentobarbitone (Sagatal) from RMB Animal Health Ltd, Dagenham; anti-insulin serum from Linco Research, St. Louis, U.S.A.; [^{125}I]-insulin from Amerlite Diagnostics, Amersham; [U-^{14}C]-sucrose, 621 mCi mmol^{-1}, and 2-deoxy-D-[1-^{3}H]-glucose, 14.4 Ci mmol^{-1} from Amersham International, Amersham.

Statistical analysis

Data are presented as mean ± s.e.mean. Data were evaluated for the effect of metformin by one-way analysis of variance, and differences between individual groups were compared by Student's unpaired t test. Differences were considered to be significant if $P<0.05$.

Results

Blood flow

Blood flow measurements were undertaken in fasted anaesthetized rats by use of transonic flow sensors around the HPV and lower abdominal IVC. Under conditions of basal glycaemia, blood flow rates were about 60 and 40 ml min^{-1} kg^{-1} for the HPV and IVC respectively. Flow rates in the HPV were temporarily (5–10 min) increased by about 10% when hyperglycaemia (10–15 mmol l^{-1}) was induced by intravenous administration of glucose.

Metformin (250 mg kg^{-1}, intrajejunally) did not significantly alter blood flow rates in either the HPV or IVC under basal or hyperglycaemic conditions.

Hyperglycaemic hyperinsulinaemic infusion

The infusion of glucose and insulin together with adrenaline and propranolol in fasted anaesthetized rats created a steady hyperglycaemia (about 11 mmol l^{-1}) and hyperinsulinaemia (about 5 ng ml^{-1}) between 90 and 180 min as shown in Figure 1. Administration of metformin (250 mg kg^{-1}, intrajejunally) at 90 min produced a progressive fall in the venous plasma glucose concentration, whereas plasma insulin was not significantly altered.

An indication of the importance of the intestine and the hind limbs as sites of metformin-stimulated glucose disposal was obtained by comparing the plasma glucose concentration in blood samples taken from the aorta (A), lower abdominal IVC and HPV at the end of the infusion period (180 min). As shown in Table 1, the arterial plasma glucose concentrations of control and metformin-treated rats were not significantly different. The metformin-treated rats showed a slightly greater fall in plasma glucose in the IVC (A-IVC) compared with control rats. The fall in plasma glucose in the HPV (A-HPV) was more pronounced in the metformin-treated rats, implicating the intestine as an important site of glucose utilization in these rats. Taking account of blood flow rates, it was estimated that glucose utilization by the intestine (HPV drainage) was 69% greater in metformin-treated rats (194.5 ± 20.3 μmol min^{-1} kg^{-1}) than controls (114.8 ± 8.5 μmol min^{-1} kg^{-1}; $P<0.01$). Glucose disposal by the hind limb region (drainage into the lower abdominal IVC) was 40% higher in metformin-treated rats (86.1 ± 8.5 μmol min^{-1} kg^{-1}) than controls (60.6 ± 8.5 μmol min^{-1} kg^{-1}; $P<0.05$).

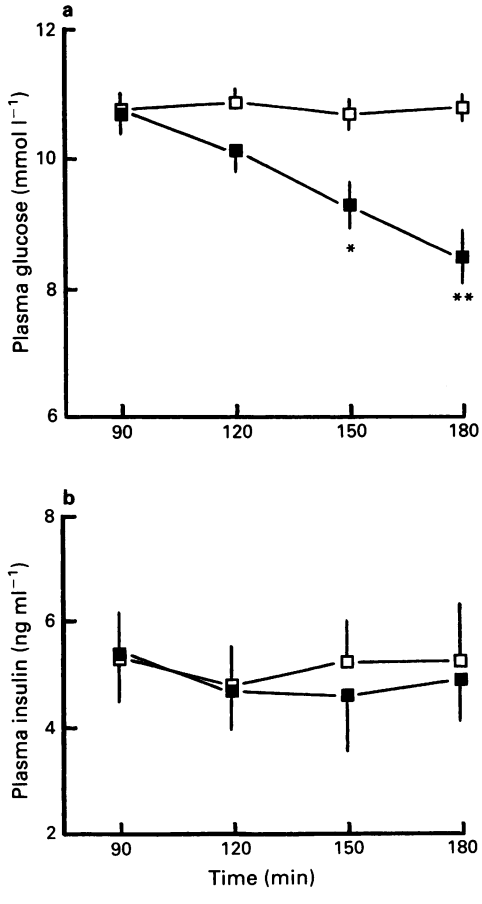

Figure 1 Plasma glucose (a) and insulin (b) concentrations of fasted anaesthetized rats during a hyperglycaemic hyperinsulinaemic infusion. At time 90 min, when a steady hyperglycaemia had been achieved, either 250 mg kg^{-1} metformin (■) or saline as control (□) was given intrajejunally. Values are mean with s.e.mean, $n=6$. *$P<0.05$, **$P<0.01$ versus control, Student's unpaired t test.

Tissue uptake of 2-deoxy-D-glucose

Tissue accumulation of 2-deoxy-D-glucose phosphate (2DGP) was measured during an IVGTT in fasted anaesthetized rats treated with metformin (250 mg kg^{-1}, intrajejunally) at 60 min before the i.v. glucose injection. Intravenous glucose tolerance was improved in the metformin-treated rats as shown in the inset to Figure 2, confirming previous experiments (Bailey & Mynett, 1994). The metformin-treated rats showed higher mean values for 2DGP accumulation into the muscle and fat, with a significant increase in 2DGP accumulation by soleus muscle. This is consistent with increased glucose utilization by the hind limb region during the hyperglycaemic hyperinsulinaemic infusion experiments. A substantial increase in 2DGP accumulation occurred in the mucosa of jejunum (by 30%) and the mid-small intestine (by 60%) of the metformin-treated rats. However, 2DGP accumulation by tissues from other regions of the gastrointestinal tract, and by brain and liver was not significantly altered.

As metformin passes down the intestine it initially produces a high concentration of the drug in the wall of the jejunum, and subsequently in the wall of ileum (Wilcock & Bailey, 1994). During the time course of the present study (up to 90 min), intrajejunally administered metformin increased 2DGP accumulation in the jejunum and mid-small intestine, but not in the ileum. Intravenous administration of metformin produces a more even distribution of drug along the length of the small intestine, although the absolute levels achieved are lower than after enteral administration (Wilcock & Bailey, 1993). Thus the tissue accumulation of 2DGP was determined after i.v. administration of metformin (250 mg kg^{-1}) at 60 min before i.v. glucose injection. The i.v. administration of metformin reduced basal plasma concentrations, and improved i.v. glucose tolerance as shown in the inset to Figure 3. After i.v. metformin, there was no significant change in 2DGP accumulation by muscle and fat. However, 2DGP accumulation in the intestinal mucosa was increased in each of the three regions examined: in the jejunum (by 20%), in the mid-small intestine (by 105%) and in the ileum (by 64%).

Table 1 Effect of metformin (250 mg kg^{-1}, intrajejunally at 90 min) on plasma glucose concentrations in aorta (A), lower abdominal inferior vena cava (IVC) and hepatic portal vein (HPV) of rats at termination (180 min) of a hyperglycaemic hyperinsulinaemic infusion

	Plasma glucose (mmol l^{-1})	
	Control $n = 6$	*Metformin* $n = 6$
Aorta	11.4 ± 0.3	10.07 ± 0.3
IVC	10.0 ± 0.4	8.7 ± 0.4*
HPV	9.7 ± 0.3	7.5 ± 0.5**
A-IVC	1.4 ± 0.1	2.0 ± 0.2*
A-HPV	1.7 ± 0.1	3.2 ± 0.2**
IVC-HPV	0.35 ± 0.1	1.2 ± 0.1**

*$P<0.05$, **$P<0.01$.

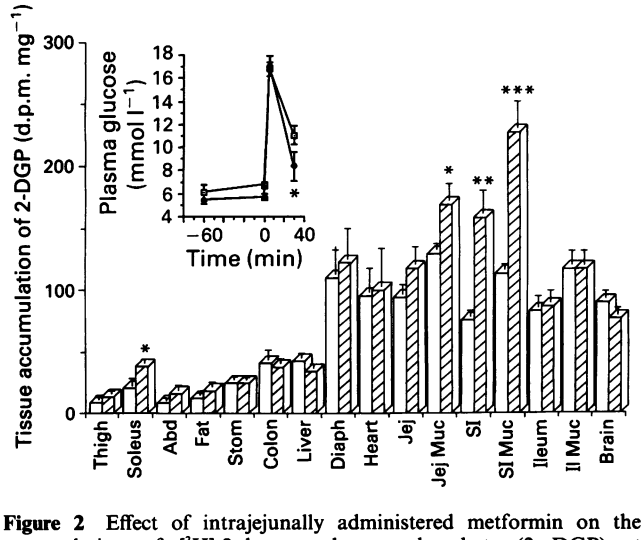

Figure 2 Effect of intrajejunally administered metformin on the accumulation of [^{3}H]-2-deoxy-D-glucose phosphate (2 DGP) at 30 min during an intravenous glucose tolerance test in fasted anaesthetized rats. At time −60 min, 250 mg kg^{-1} metformin (hatched columns) or saline as control (open columns) was given intrajejunally. Inset shows plasma glucose concentrations during the glucose tolerance test: metformin (■), control (□). The rate of glucose disappearance (% min^{-1}) was 1.7 ± 0.3 and 2.5 ± 0.3 for control and metformin-treated rats respectively. Values are mean with s.e.mean, $n = 6$. *$P<0.05$, **$P<0.01$, ***$P<0.001$ versus control, Student's unpaired t test.

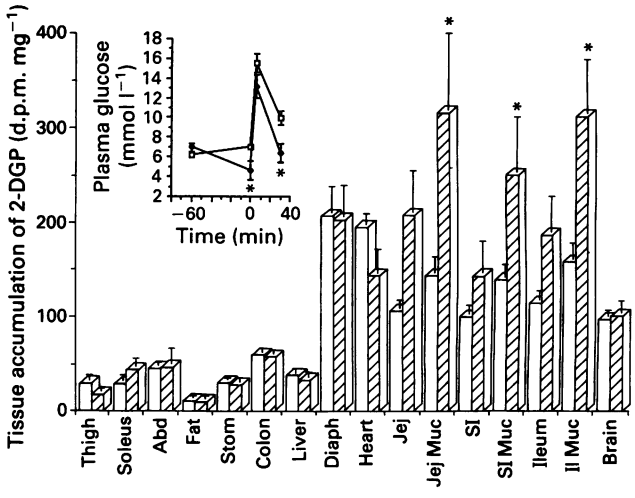

Figure 3 Effect of intravenously administered metformin on the accumulation of [^{3}H]-2-deoxy-D-glucose phosphate (2DGP) at 30 min during an intravenous glucose tolerance test in fasted anaesthetized rats. At time −60 min, 250 mg kg^{-1} metformin (hatched columns) or saline as control (open columns) was given intravenously. Inset shows plasma glucose concentrations during the glucose tolerance test: metformin (■), control (□). The rate of glucose disappearance (% min^{-1}) was 1.7 ± 0.3 and 2.5 ± 0.2 for control and metformin-treated rats respectively. Values are mean with s.e.mean, $n = 6$. *$P<0.05$ versus control, Student's unpaired t test.

Discussion

The ability of metformin to enhance insulin-mediated glucose disposal is well established (Nosadini *et al.*, 1987; DeFronzo *et al.*, 1991; Riccio *et al.*, 1991), and skeletal muscle has been implicated as an important destination of that glucose (Frayn & Adnitt, 1972; Bailey & Puah, 1986; Rossetti *et al.*, 1990). Recent studies have indicated that the intestine is also an important site of metformin-stimulated glucose utilization (Penicaud *et al.*, 1989; Wilcock & Bailey, 1990; Bailey *et al.*, 1992). However, a quantitative determination of the effect of metformin on glucose utilization by the intestine *in vivo* has not been reported.

Glucose utilization is sensitive to changes in regional blood flow (DeFronzo *et al.*, 1981), and it has been claimed that metformin may alter hepatic blood flow (Ohnhaus *et al.*, 1978). Since 70–80% of hepatic blood flow derives from the intestinal drainage into the hepatic portal vein (Greenway & Stark, 1971), it was pertinent to check the effect of metformin on blood flow in this vessel. Measurements of portal blood

flow in laboratory rodents which were hitherto estimated indirectly (Greenway & Stark, 1971; Aardal *et al.*, 1978), can now be determined directly with transonic flow sensors (Drost *et al.*, 1984).

A previous study, using internal calorimetry with thermocouples implanted into the liver, suggested that a high dose of metformin (500 mg kg^{-1}) increased liver blood flow by 10–25% over 2 h but a lower dose (175 mg kg^{-1}) had no effect (Ohnhaus *et al.*, 1978). Using the transonic flow sensors we noted that a rise in plasma glucose produced a small increase in blood flow from the intestine (Abumrad *et al.*, 1982), but there was no effect of metformin (250 mg kg^{-1}) on blood flow in the HPV or lower abdominal IVC under conditions of basal or raised glycaemia.

During the hyperglycaemic hyperinsulinaemic infusion, glucose utilization by the hind limb region (about 60 μmol min^{-1} kg^{-1}) was comparable with, but predictably slightly higher than, glucose utilization during euglycaemic hyperinsulinaemic clamp studies (Brichard *et al.*, 1992). The 40% greater utilization of glucose by the hind limb region of metformin-treated rats is also consistent with clinical estimates of the effect of metformin on whole body glucose utilization during hyperinsulinaemic glucose clamp procedures in diabetic patients (Nosadini *et al.*, 1987; DeFronzo *et al.*, 1991; Riccio *et al.*, 1991).

Our *in vivo* estimate of glucose utilization by the intestine (drainage into HPV) of rats during the hyperglycaemic hyperinsulinaemic infusion studies (about 115 μmol min^{-1} kg^{-1}) was higher than estimated in the hyperinsulinaemic state in dogs (Abumrad *et al.*, 1982; Barrett *et al.*, 1985) and during *in vitro* gut perfusion studies in rats (Windmueller & Spaeth, 1971). This may partly reflect the combination of hyperglycaemia and hyperinsulinaemia which enhances intestinal glucose utilization (Barrett *et al.*, 1982), and increased intestinal blood flow during pentobarbitone anaesthesia (Aardal *et al.*, 1978). Clinical studies have not discriminated glucose utilization by the intestine from net glucose exchange by all splanchnic tissues due to the difficulty of hepatic portal cannulation (Ferrannini *et al.*, 1985).

The 69% greater utilization of glucose by the intestine of metformin-treated rats suggests that metformin exerts a stronger effect on glucose utilization by this tissue than the predominantly skeletal muscle of the hind limb region. This is supported by the 2DGP accumulation studies showing that metformin produced a greater increase in 2DG uptake by tissues of the small intestine than by skeletal muscles. The intestinal effect of metformin was exerted mainly on the mucosa, and was evident in the jejunum and mid-small intestine after intrajejunal administration of the drug. However, after i.v. administration of metformin, which gives a more even distribution of the drug along the small intestine (Wilcock & Bailey, 1994), 2DG uptake was also increased in ileal mucosa.

Since the intestinal mucosa accumulates much higher concentrations of metformin (up to 10^{-3} mol kg^{-1}) compared with other tissues such as skeletal muscle (in the range of 10^{-5} mol kg^{-1}), it is possible that the drug acts differently on the intestinal tissue (Wilcock & Bailey, 1994). Whereas therapeutic doses of metformin do not significantly increase lactate production by skeletal muscle (Bailey & Puah, 1986; Jackson *et al.*, 1987), there is a substantial increase in lactate production by the intestine (Wilcock & Bailey, 1990; Bailey *et al.*, 1992). Thus the exposure of intestinal tissue to very high concentrations of metformin may stimulate anaerobic glycolysis, while lower concentrations of the drug in skeletal muscle enhance mainly glycogenesis and glucose oxidation (Bailey & Puah, 1986; Rossetti *et al.*, 1990; Reddi & Jyothirmayi, 1992). Preliminary evidence suggests that metformin increases glucose uptake in muscle and fat by increasing the translocation of the glucose transporter GLUT1, and possibly GLUT4, into the plasma membrane (Matthaei *et al.*, 1991; Sarabia *et al.*, 1992). Since these particular isoforms of the transporter are not expressed (to any extent) in the intestinal mucosa (Pessin & Bell, 1992) the mechanism through which metformin promotes glucose uptake in this tissue remains to be determined. Although the presence of some insulin is required for normal glucose metabolism by the intestine, the insulin dose-response effect is different from muscle and fat (Kellet *et al.*, 1984). This supports the possibility that metformin may enhance glucose utilization in the intestine through a different mechanism from that which appears to operate in muscle and fat (Bailey, 1993).

The contention that very high concentrations of metformin in the intestine may compromise ATP formation locally has not been examined, despite the evidence that metformin increases intestinal lactate production. A significant proportion of this lactate is extracted by the liver under conditions of basal glycaemia (Bailey *et al.*, 1992). There is also an adjustment of lactate metabolism after a glucose challenge which results in net lactate utilization by the periphery (Jackson *et al.*, 1987; Bailey *et al.*, 1992). Thus it is likely that metformin-stimulated glucose utilization by the intestine makes an important contribution to increased glucose recycling (Penicaud *et al.*, 1989). This is envisaged to include an intrasplanchnic substrate cycle in which glucose is converted to lactate in the intestine, released into the portal circulation and extracted by the liver to form glucose (Bailey, 1993).

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