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Effect of metformin on glucose metabolism in the splanchnic bed

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1 Use of the antihyperglycaemic agent, metformin, is often associated with a small rise in circulating lactate. This study investigates the source of the lactate and examines the effect of metformin on glucose metabolism by the intestine and liver of rats.

2 Changes in plasma glucose and lactate were measured in the inferior vena cava (IVC), hepatic portal vein (HPV), hepatic vein (HV) and aorta (A) after intrajejunal administration of metformin (50 and 250 mg kg^{-1}) without and with glucose (2 g kg^{-1}).

3 Metformin 250 mg kg^{-1} reduced the hyperglycaemic response to a glucose challenge, associated with a greater reduction of glucose concentrations in the HPV (average decrease of 33% at 60 and 120 min) than at other sites.

4 Both doses of metformin increased lactate concentrations in the glucose-loaded state: the highest concentration (2.5 fold increase) was recorded in the HPV 60 min after administration of 250 mg kg^{-1} metformin, with a high lactate concentration persisting in the HV at 120 min. Metformin 250 mg kg^{-1} also increased lactate concentrations in the basal state, with highest concentrations (2 fold increase) in the HPV.

5 Two hours after intrajejunal administration of metformin, 50 mg kg^{-1}, rings of tissue from the small intestine showed an average 22% decrease in glucose oxidation ([^{14}C]-glucose conversion to $^{14}CO_2$) and a 10% increase in lactate production. Since glucose metabolism in the gut is predominantly anaerobic, metformin caused an overall 9.5% increase of intestinal glucose utilization.

6 Metformin, 10^{-6} and 10^{-4} mol l^{-1}, did not significantly alter glucose oxidation or lactate production by isolated hepatocytes, but a very high concentration of metformin (10^{-2} mol l^{-1}) increased lactate production by 60%.

7 The results support the view that metformin increased intestinal glucose utilization and lactate production by the intestine. Under basal conditions there was net extraction of lactate by the liver but not after an enteral glucose load.

Keywords: Metformin; glucose metabolism; lactate; splanchnic bed; liver; intestine

Introduction

Metformin (dimethylbiguanide) is an antihyperglycaemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM) (Bailey, 1988; Bailey & Nattrass, 1988). The glucose-lowering action is associated with increased peripheral glucose disposal, particularly by skeletal muscle, and decreased hepatic glucose production (Bailey & Puah, 1986; Prager *et al.*, 1986; Nosadini *et al.*, 1987; Wollen & Bailey, 1988). There is also evidence that metformin decreases the rate of intestinal glucose absorption (Lorch, 1971; Wilcock & Bailey, 1990a). The antihyperglycaemic action of metformin requires the presence of insulin but the drug does not stimulate insulin secretion (Bailey, 1988; Bailey & Nattrass, 1988).

Concern about the use of metformin has focused on its propensity to raise circulating lactate concentrations, mainly after meals, although the magnitude of this effect is generally small (<2 mmol l^{-1}) (Campbell *et al.*, 1987; Jackson *et al.*, 1987; Bailey, 1988; Bailey & Nattrass, 1988).

Metformin does not cause lactic acidosis if appropriately prescribed, i.e. if patients with renal and hepatic insufficiency are excluded (Bailey, 1988; Bailey & Nattrass, 1988; Campbell, 1990). Extra lactate production during metformin therapy was presumed to arise from peripheral tissues such as skeletal muscle, but recent studies failed to confirm that notion (Bailey & Puah, 1986; Jackson *et al.*, 1987).

The present study investigates the possibility that increased lactate production by metformin arises from the splanchnic bed. *In vivo* and *in vitro* experiments in rats have been undertaken to examine the effect of metformin on glucose metabolism and lactate production by the intestine and liver.

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Methods

Animals

Adult male Wistar rats weighing about 200 g were maintained in an air-conditioned room at 22 ± 2°C with 12 h light (08 h 00 min–20 h 00 min), and supplied a standard pellet diet (Rat breeding diet, Heygate and Sons, Northampton) and tap water.

Blood sampling studies

Rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.) and maintained under anaesthesia with further doses of 15 mg kg^{-1} h^{-1}. Rectal temperature was held at 34–36°C. The abdomen was opened and blood samples (50 µl) were taken through fine heparinised needles inserted at 4 sites: aorta (A) immediately anterior to the branching of the iliac arteries; inferior vena cava (IVC) adjacent to the right ilio-lumbar vein; hepatic portal vein (HPV) immediately before branching into the liver; and hepatic vein (HV) as close to the liver as possible. Test substances were administered by injection into the second loop of the jejunum and massaged distally along the intestine. Groups of 12 h-fasted

rats received either saline (0.9% NaCl, 5 ml kg^{-1}) or metformin hydrochloride (50 and 250 mg kg^{-1} 5 ml^{-1}). Groups of 4 h fasted rats received either glucose (2 g kg^{-1} 5 ml^{-1}) or glucose with metformin hydrochloride (doses as above). Blood samples were taken from all sites immediately before and at 60 and 120 min after administration of test substances. Plasma glucose (Stevens, 1971) and lactate (Noll, 1974) were determined.

Intestinal rings

Fed rats were anaesthetized and treated by intrajejunal administration of either saline or metformin hydrochloride (50 mg kg^{-1}) as above. After 2 h, rings of tissue (about 30 mg) were prepared from the proximal, middle and distal regions of the jejunum and ileum. The rings were washed in incubation buffer and incubated for 2 h at 37°C in 3 ml of pregassed (95% O_2:5% CO_2) Krebs Ringer bicarbonate (KRB) buffer, pH 7.4, containing bovine serum albumin 20 mg ml^{-1}, glucose 10 mmol l^{-1}, D-[U-^{14}C]-glucose 0.5 μCi ml^{-1} and insulin 10^{-8} mol l^{-1}. Production of $^{14}CO_2$ and lactate was determined (Bailey & Puah, 1986).

Hepatocytes

Hepatocytes were isolated from anaesthetized fed rats by a modification of the collagenase method (Berry & Friend, 1969). The following four buffers were infused at 5 ml min^{-1} into the HPV without recirculation: calcium-free KRB supplemented with EGTA (0.5 mmol l^{-1}) and sodium heparin (2 units ml^{-1}) for 5 min; calcium-free KRB for 5 min; KRB supplemented with collagenase (0.5 mg min^{-1}) for 10 min; and KRB supplemented with bovine serum albumin (20 mg ml^{-1}) and glucose (10 mmol l^{-1}) for 5 min. Buffers were pH 7.4, saturated with 95% O_2:5% CO_2 and infused at 37°C. The liver was removed and cells were separated by disruption with dissecting needles. The suspension was filtered through muslin, washed and preincubated for 15 min at 37°C in pregassed (95% O_2:5% CO_2) buffer as used for the last infusion step of the hepatocyte isolation. Cell viability assessed by 0.1% trypan blue exclusion was accepted at >90%, and the number of viable cells was determined. Test incubations were performed with a suspension of 7×10^6 viable cells ml^{-1} in a final volume of 1.5 ml. Test buffer was the same as for preincubation with the addition of 1.0 μCi ml^{-1} D-[U-^{14}C]-glucose, without and with insulin (10^{-8} mol l^{-1}) and metformin (10^{-6}–10^{-2} mol l^{-1}). Production of $^{14}CO_2$ and lactate was measured as above after incubation for 2 h at 37°C.

Chemicals

Crystalline bovine insulin (24.3 iu mg^{-1}), bovine serum albumin (fraction V, RIA grade), EGTA and collagenase (type IV, from *Clostridium histolyticum*) were from Sigma Chemical Company, Poole, Dorset; D-[U-^{14}C]-glucose (specific activity 270 mCi $mmol^{-1}$) was from Amersham International, Amersham, UK; and pure metformin hydrochloride (batch 2452) was from Lipha Pharmaceuticals, West Drayton.

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 *t* test with Bonferroni's correction for multiple comparison. Differences were considered to be significant if $P < 0.05$.

Results

Blood sampling studies

Intrajejunal administration of saline (5 ml kg^{-1}), or metformin (50 and 250 mg kg^{-1}) to 12 h-fasted anaesthetized rats did not significantly alter plasma glucose concentrations at each of the four sites sampled (IVC, HPV, HV and A) over

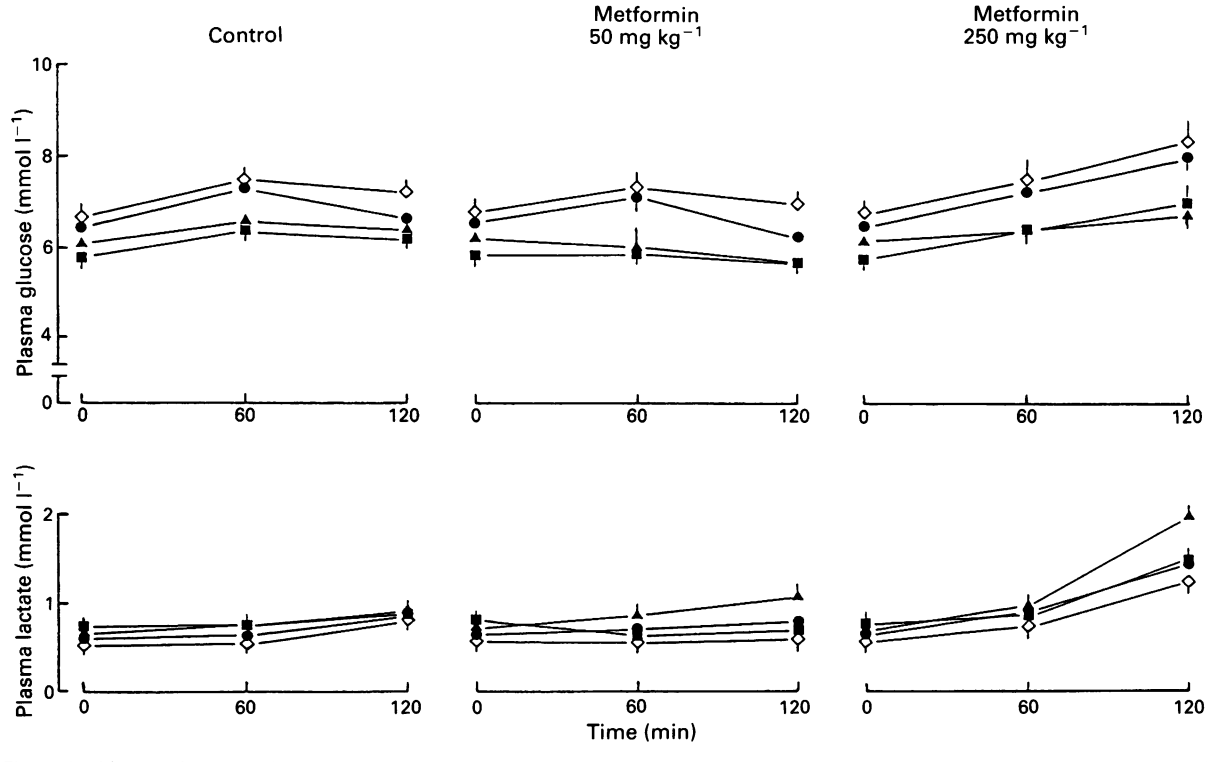

Figure 1 Plasma glucose and lactate concentrations of 12 h-fasted anaesthetized rats after intrajejunal administration of saline, and 50 and 250 mg kg^{-1} metformin. (■) Inferior vena cava; (▲) hepatic portal vein; (◇) hepatic vein; (●) aorta. Values are mean with s.e.mean shown by vertical lines, $n = 6$.

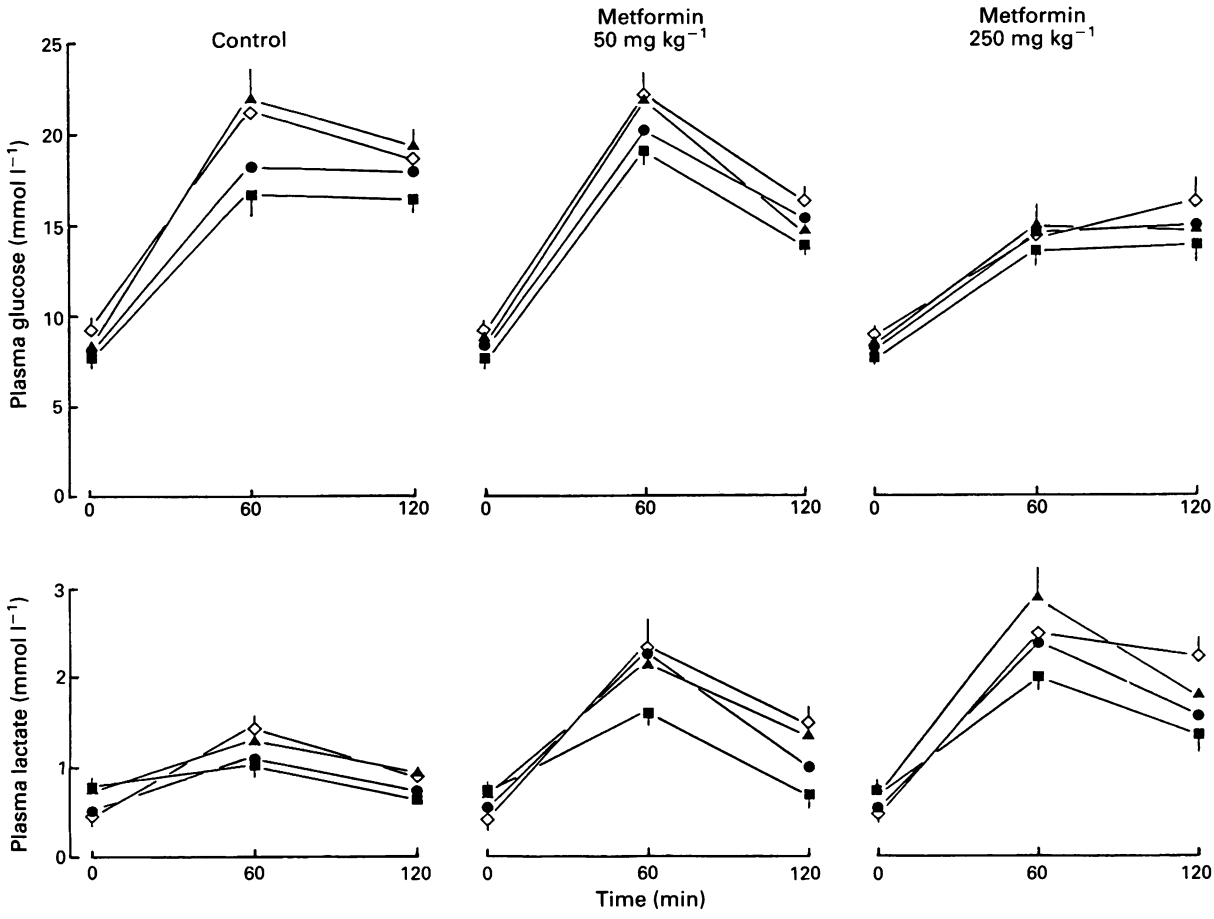

Figure 2 Plasma glucose and lactate concentrations of 4 h-fasted anaesthetized rats after intrajejunal administration of glucose (2 g kg^{-1}) without and with 50 and 250 mg kg^{-1} metformin. (■) Inferior vena cava; (▲) hepatic portal vein; (◇) hepatic vein; (●) aorta. Values are mean with s.e.mean shown by vertical lines, $n = 10$ for controls and $n = 5$ for the metformin-treated groups.

the 120 min duration of the study (Figure 1). Plasma lactate concentrations at each of the four sites were not significantly altered by administration of saline or metformin, 50 mg kg^{-1}. However, 250 mg kg^{-1} metformin increased plasma lactate concentrations (ANOVA, $P<0.05$), with the greatest increase occurring in the HPV (by 104%, $P<0.01$) at 120 min.

Intrajejunal administration of glucose (2 g kg^{-1}) increased plasma glucose concentrations at all four sites sampled (Figure 2). The greatest increase occurred in the HPV and the smallest increase in IVC as expected. Each dose of metformin exerted an antihyperglycaemic effect but the pattern of the glucose response was different. Administration of 50 mg kg^{-1} metformin with the glucose, reduced plasma glucose concentrations at 120 min in HPV (by 38%, $P<0.01$) and IVC (by 17%, $P<0.05$). When 250 mg kg^{-1} metformin was administered with the glucose, plasma glucose concentrations were reduced at 60 min at all four sites (IVC by 21%, HPV by 37%, HV by 36% and A by 17%, all $P<0.05$), and at 120 min in HPV (by 29%, $P<0.05$). Plasma lactate concentrations were raised at all four sites at 60 min after intrajejunal glucose administration (IVC by 46%, HPV by 77%, HV by 270% and A by 121%, all $P<0.05$ compared with time zero). In glucose-loaded rats, metformin increased lactate concentrations in a dose-dependent manner. Administration of 50 mg kg^{-1} metformin with glucose produced greater increases in plasma lactate than glucose alone at all four sites at 60 min (IVC by 48%, HPV by 52%, HV by 58% and A by 113% greater than glucose only, all $P<0.05$). Administration of 250 mg kg^{-1} metformin with the glucose increased plasma lactate at all four sites at 60 and 120 min: at 60 min IVC by 99%, HPV by 123%, HV by 62% and A by 118% greater than glucose only; at 120 min IVC by 103%, HPV by 89%, HV by 160% and A by 99% greater than glucose only. All values were significantly ($P<0.05$) greater than glucose alone or glucose with 50 mg kg^{-1} metformin. It is noteworthy that 250 mg kg^{-1} metformin caused a protracted increase in plasma lactate in the HV of glucose-loaded rats.

Intestinal rings

Two hours after intrajejunal administration of 50 mg kg^{-1} metformin, rings of proximal, middle and distal regions of the jejunum and ileum were prepared and incubated *in vitro* with 10 mmol l^{-1} U-[^{14}C]-glucose. Glucose oxidation assessed by production of $^{14}CO_2$, was decreased (by 19–38%) in the mid-jejunum through to the mid-ileum (Figure 3). Lactate production was increased (by 21–25%) in the mid-jejunum through to the proximal ileum. Since intestinal glucose metabolism was mainly anaerobic, metformin increased glucose utilization by 23% in the mid-jejunum to proximal ileum, and by 9.5% for the overall jejunum and ileum.

Hepatocytes

Glucose oxidation and lactate production were assessed using hepatocytes of fed rats incubated with metformin (10^{-6}, 10^{-4} and 10^{-2} mol l^{-1}). Metformin 10^{-6} and 10^{-4} mol l^{-1} did not significantly alter glucose oxidation or lactate production, either in the absence or presence of 10^{-8} mol l^{-1} insulin, although 10^{-4} mol l^{-1} metformin increased mean values for lactate production (Figure 4). Metformin, 10^{-2} mol l^{-1}, decreased glucose oxidation (about 30%) and increased lactate production (about 60%) in the absence and presence of 10^{-8} mol l^{-1} insulin. Insulin (10^{-8} mol l^{-1}) alone did not significantly alter glucose oxidation or lactate production.

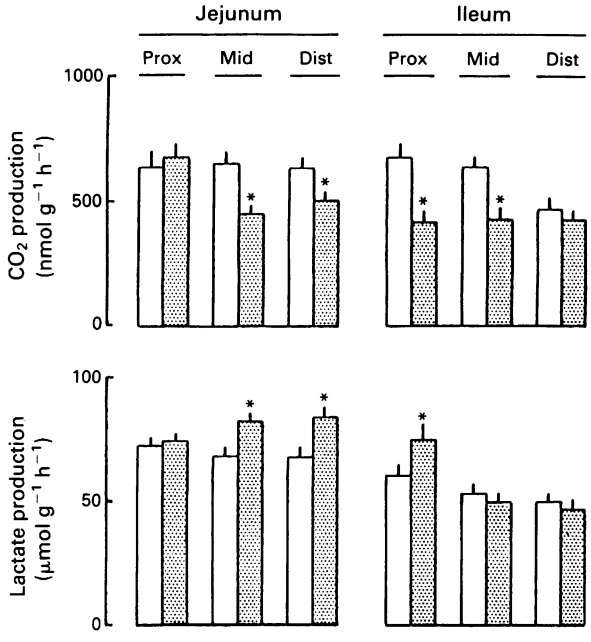

Figure 3 Glucose oxidation to CO_2 and lactate production by intestinal rings prepared from rats 2 h after intrajejunal administration of 50 mg kg^{-1} metformin. Open columns indicate controls; stippled columns indicate metformin-treated. Values are mean with s.e.mean shown by vertical lines, $n = 6$. $^*P < 0.05$ versus control (Student's t test).

Discussion

Clinically, metformin is prescribed in doses of 500–1000 mg twice or thrice daily with meals. Thus 50 mg kg^{-1} metformin given to a rat is equivalent on a weight related basis to the maximum clinical daily dose (3 g 60 kg^{-1}) delivered as a single bolus. In man, consumption of 1 g metformin produces peak peripheral plasma concentrations of $1-5 \times 10^{-5}$ mol l^{-1} at 1–2 h (Pentikainen *et al.*, 1979; Tucker *et al.*, 1981). In rats, an oral bolus of 50 mg kg^{-1} metformin produced similar maximum concentrations of metformin in peripheral plasma after 1 h (Wilcock *et al.*, 1991). Since rats are less sensitive to the antihyperglycaemic effect of metformin than man (Sterne, 1969), the present selection of metformin doses (50 and 250 mg kg^{-1}) and concentrations (10^{-6}–10^{-2} mol l^{-1}) was considered appropriate to investigate therapeutic and supratherapeutic effects.

Unlike sulphonylureas, metformin is not able alone to cause clinical hypoglycaemia, and it has little effect on basal glucose concentrations in the non-diabetic state (Bailey, 1988; Bailey & Nattrass, 1988; Campbell, 1990). However, metformin lowers glucose concentrations in NIDDM patients and in the glucose-loaded non-diabetic state, hence its designation as an 'antihyperglycaemic' agent. Accordingly, metformin lowered the plasma glucose response to an intrajejunal glucose challenge in the present study. Metformin 250 mg kg^{-1} most strongly reduced HPV glucose concentrations consistent with decreased intestinal glucose absorption (Lorch, 1971; Wilcock & Bailey, 1990a). Recent studies have shown that metformin increases glucose utilization by the intestine (Penicaud *et al.*, 1989; Wilcock & Bailey, 1990b), and our preliminary *in vitro* data indicated a concomitant increase in lactate production (Wilcock & Bailey, 1990b). Although the measurement of lactate concentrations at different sites does not provide information on lactate turnover, the present observation that 250 mg kg^{-1} metformin produced the highest lactate concentrations in the HPV, in both the basal and glucose-loaded state, substantiates the theory that metformin can increase intestinal net lactate production independently of intestinal glucose absorption (Wilcock & Bailey, 1990b). However, the effect of metformin on net lactate production was greater during a glucose challenge, although the absolute magnitude of the increase in lactate concentrations was always small (maximum increase 2.5 fold in the HPV).

In the basal state lactate concentrations were consistently higher in the HPV than HV, suggesting net extraction of lactate by the liver, which would buffer the extent of change in peripheral lactate concentrations. During a glucose challenge the concentration of lactate in the HV may exceed that in the HPV, as evident at 120 min after 250 mg kg^{-1} metformin. This is consistent with other evidence that when glucose and lactate concentrations are raised in the HPV, the liver is no longer able to operate as a net lactate extractor (Jackson *et al.*, 1990). This would explain the clinical observation that metformin increases peripheral lactate concentrations mainly during meal absorption (Bailey & Nattrass,

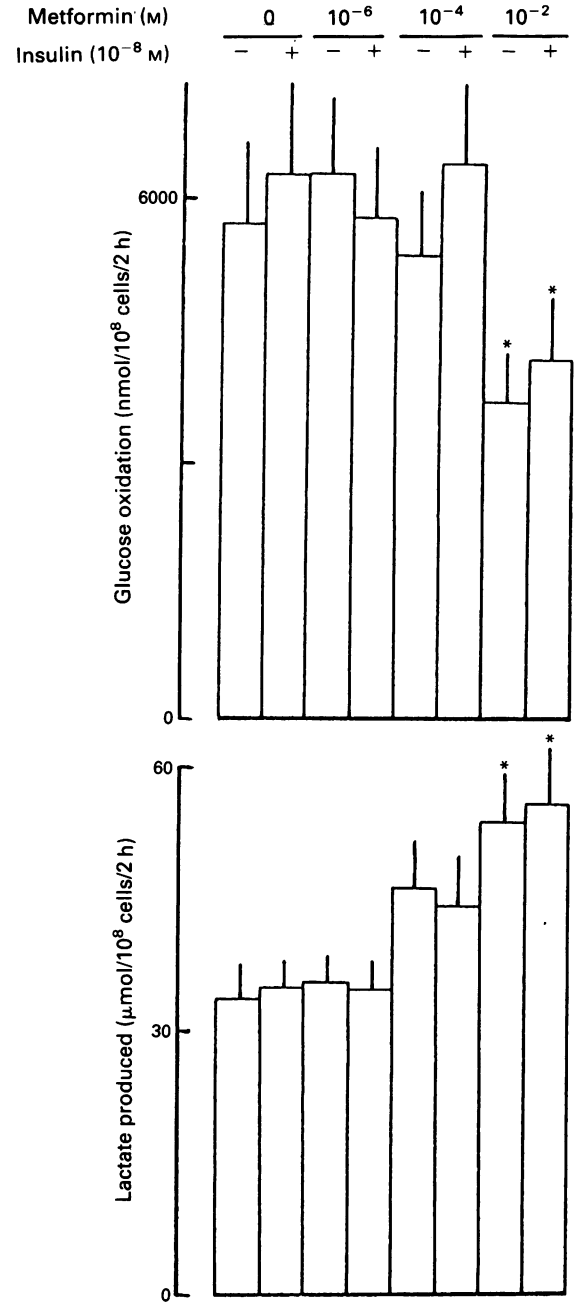

Figure 4 Glucose oxidation to CO_2 and lactate production by rat hepatocytes incubated for 1 h with metformin (10^{-6}–10^{-2} mol l^{-1}) in the absence and presence of insulin (10^{-8} mol l^{-1}). Values are mean with s.e.mean shown by vertical lines, $n = 8$. $^*P < 0.05$ versus control receiving same amount of insulin (Student's t test).

1988; Campbell, 1990), and is consistent with a recent clinical study showing that a net increase in lactate production after metformin does not come from the periphery (Jackson *et al.*, 1987). Indeed *in vitro* studies have discounted muscle, fat, brain and skin as sources of extra lactate production by metformin (Wilcock & Bailey, 1990b). The present study excludes the periphery as the net source of the extra lactate because lactate concentrations were slightly higher in the aorta than IVC.

Although therapeutic concentrations (10^{-6}–10^{-4} mol l^{-1}) of metformin did not significantly alter glucose oxidation or lactate production by hepatocytes, 10^{-4} mol l^{-1} metformin consistently increased mean values for lactate production. Moreover, a very high concentration of metformin (10^{-2} mol l^{-1}) reduced glucose oxidation and increased lactate production by hepatocytes, consistent with the measurement of highest glucose and lactate concentrations in the HV at 120 min after treatment of glucose-loaded rats with 250 mg kg^{-1} metformin. The effect of a high metformin concentration in reducing glucose oxidation and increasing lactate production by hepatocytes is comparable with the effect of the drug on the small intestine, but contrasts with the effect of lower (therapeutic) concentrations of metformin that enhance glucose oxidation by peripheral insulin-sensitive tissues, especially in mildly diabetic states (Frayn & Adnitt, 1972; Bailey & Puah, 1986; Wilcock & Bailey, 1990b).

Interestingly, rats appear to be more sensitive than mice to the effects of metformin on glucose metabolism in the intestine and liver; indeed even 10^{-2} mol l^{-1} metformin did not significantly alter glucose oxidation by these tissues from mice (Wilcock & Bailey, 1990b). In conclusion, the present study has provided evidence compatible with the view that the main source of extra lactate produced by metformin is the intestine. This is relatively small and may be extracted by the liver unless the liver is presented with high concentrations of both lactate and glucose.

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