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Inhibitory Effect of Metformin on Intestinal Glucose Absorption in the Perfused Rat Intestine

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ABSTRACT. To investigate the effect of metformin on intestinal glucose absorption, a perfusion study of the intestine was performed in the rat. Male Wistar albino rats (8 weeks old) were used in the present study. The glucose absorption by the perfused intestine (788.1 ± 81.9 μmol/30 min) was not changed significantly by the direct addition of metformin (90 μg/mL) to the perfusing medium (737.0 ± 118.2 μmol/30 min) or by intraduodenal metformin (250 mg/kg in saline solution) infusion (772.8 ± 106.3 μmol/30 min). In rats orally administered metformin (250 mg/kg) for 5 days, glucose absorption by the perfused intestine (375.0 ± 164.3 μmol/30 min) was significantly ($P < 0.001$) lower than that in control rats (811.0 ± 83.1 μmol/30 min). These results indicate that metformin had a significant effect on the digestive tract, and that metformin treatment exerted an inhibitory effect on intestinal glucose absorption in the rat. BIOCHEM PHARMACOL **59**;7:887–890, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. absorption; glucose; intestine; metformin; perfusion; rat

Metformin is a biguanide that has been used to treat type 2 diabetes mellitus in humans for many years [1, 2]. Although the mode of action of metformin is incompletely understood, many studies have demonstrated that metformin does decrease hepatic glucose production *in vivo* and *in vitro* [3–7], and several studies have suggested an additional role in improving peripheral insulin sensitivity in humans and rats [8–11]. It has been reported that the greatest accumulation of metformin occurs in tissues of the small intestine after oral administration [12]. Several studies have shown that metformin decreases glucose transport by the intestine, and this may contribute to the blood glucose-lowering effect of the drug [13–15]. However, little is known about the effect of metformin on intestinal glucose absorption.

To elucidate these issues, the effect of metformin on intestinal glucose absorption was investigated in the perfused intestine of the rat.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (8 weeks old) were used in the present study. Metformin (1,1-dimethyl biguanide, from the Sigma Chemical Co., 250 mg/kg body weight in 0.9% NaCl solution) was administered through a gastric catheter once daily for a period of 5 days. The controls received 0.9% NaCl solution alone. The caloric content of the normal chow was distributed as 58% carbohydrate, 12% fat, and 30% protein. All animals were allowed free access to water. Normal food consumption was maintained during the metformin treatment. Studies were performed 5 hr after the last dose of metformin. Animals were weighed before the experiments, and anesthesia was induced intraperitoneally using 50 mg/kg of pentobarbital sodium. Then blood was drawn from the femoral vein for measurement of plasma glucose, insulin, triglyceride, and FFA§ concentrations.

Preparation of the Intestine

A modified method [16, 17] based on that of Levin *et al.* [18] was used for isolation and perfusion of the rat intestine. The pylorus was ligated and transected. The spleen was resected, and the pancreas was removed from the duodenum with minimal trauma. The superior mesenteric artery and portal vein were cannulated. The entire preparation, consisting of duodenum, jejunum, ileum, and colon, was removed and placed in the perfusion chamber. Then a polyethylene tube was inserted into the duodenum to infuse glucose or metformin solution as required, and the cecum was incised to allow the removal of intestinal content. The isolated intestine was perfused at a flow rate of 6 mL/min.

Perfusion Method

PERFUSION MEDIUM. The basal perfusion medium consisted of a Krebs–Ringer bicarbonate buffer containing

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§ *Abbreviations*: FFA, free fatty acid(s); and GLUT, glucose transporter.

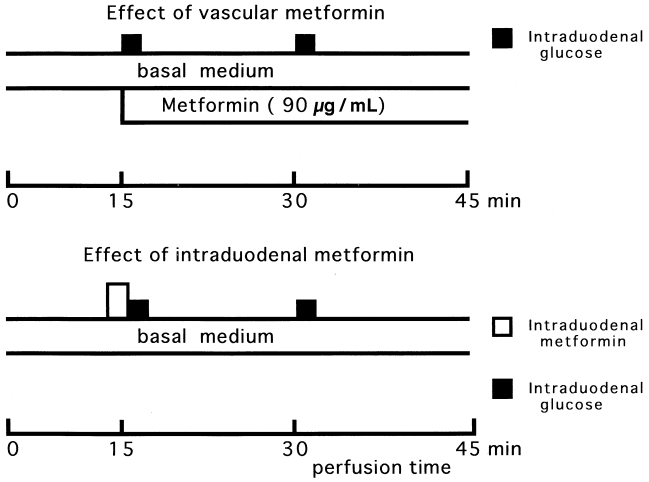

FIG. 1. Protocols of the perfusion of the intestine.

0.5% BSA, 4.6% Dextran T-70 (Green Cross Co.), and 5.5 mM glucose. To prevent glucose metabolism by erythrocytes, an erythrocyte-free medium was used in the present study. The venous effluent was collected every minute and stored at −70° until the assay was performed. During perfusion, the medium and the intestine were warmed and kept at 37°, and the medium was bubbled with a mixture of 95% O_2 and 5% CO_2. The pH was maintained at 7.4.

PERFUSION OF THE INTESTINE. The control intestines were perfused with basal medium for 45 min. Three milliliters of 10% glucose solution (dissolved in 0.9% NaCl solution) was bolus-infused intraduodenally at 15 and 30 min as shown in Fig. 1. To evaluate the direct effect of metformin on glucose absorption, after a 15-min perfusion with basal medium, the intestine was perfused with medium containing metformin (90 μg/mL) for 30 min as shown in Fig. 1. To evaluate the effect of intraluminal metformin on glucose absorption, metformin (250 mg/kg body weight in 0.5 mL saline solution) was infused intraduodenally immediately before the glucose infusion as shown in Fig. 1.

In the metformin-treated rats, the intestine was perfused with a basal medium for 45 min. Then, glucose solution was infused intraduodenally at 15 and 30 min as mentioned above. The intestines of normal, control (sham-treated), or metformin-treated rats were perfused for 45 min without intraduodenal glucose infusion, to evaluate the glucose uptake from the circulation. Isolated rat intestine perfused with the technique used here has been observed to remain viable for at least 1 hr, according to Levin *et al.* [18].

Calculations

Glucose uptake by the intestine was calculated by the following formula: [glucose infused during perfusion (15–45 min) − effluent glucose during perfusion (15–45 min)]. Glucose absorption by the intestine was calculated by the following formula: [glucose concentration in the effluent after intraduodenal glucose infusion (15–45 min) − glucose concentration in the effluent before intraduodenal glucose infusion (10–15 min)] × perfusion volume.

Histological Examination

One-centimeter sections of duodenum, jejunum, and ileum were washed with 0.9% NaCl and placed in 10% formol saline. The tissue was later stained with hematoxylin and eosin.

Measurements

Glucose concentration was measured by the glucose oxidase method [19]. Plasma triglyceride and FFA concentrations were measured enzymatically using a commercially available kit. Insulin was measured by radioimmunoassay.

Statistical Analysis

Data are expressed as means ± SD. ANOVA and two-tailed Student's *t*-test were used for statistical evaluation.

RESULTS

Characteristics of the Rats

As shown in Table 1, body weight, fasting blood glucose, plasma insulin, triglyceride, and FFA concentrations in the metformin-treated rats were not significantly different from those in the control (sham-treated) rats, respectively. The wet weight of the whole intestine (5.5 ± 0.3 g) in the sham-treated rats did not differ significantly from that (5.1 ± 0.4 g) in the metformin-treated rats.

Glucose Uptake by the Perfused Intestine

EFFECT OF INTRAVASCULAR OR INTRADUODENAL METFORMIN ON GLUCOSE UPTAKE. Glucose uptake by the perfused intestine (42.1 ± 13.2 μmol/30 min, N = 6) was not changed significantly by the addition of intravascular met-

TABLE 1. Characteristics of the rats

	Body weight (g)	Blood glucose (mg/dL)	Insulin (μU/mL)	Triglyceride (mg/dL)	FFA (μEq/L)
Control	189 ± 14	88.4 ± 10.7	15 ± 2	77 ± 10	845 ± 112
Metformin	192 ± 15	86.8 ± 10.0	14 ± 2	73 ± 8	785 ± 103

Control: sham-treated rats (N = 24). Metform: metformin-treated rats (N = 24). Values are means ± SD.

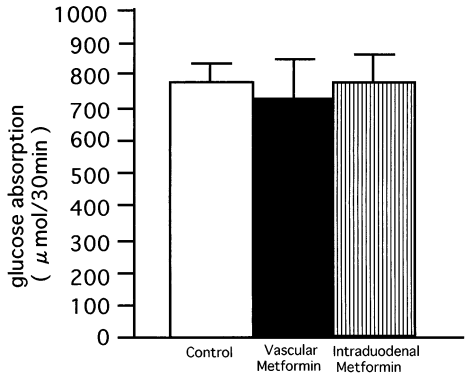

FIG. 2. Effect of intravascular or intraduodenal metformin on glucose absorption by the perfused intestine. The bars represent means ± SD. For the control group (N = 6), intestines were perfused with basal perfusion medium; for the vascular metformin group (N = 6), metformin (90 μg/mL) was added to the perfusing medium; and for the intraduodenal metformin group (N = 6), metformin (250 mg/kg in saline solution) was bolus-infused into the duodenum.

formin (39.6 ± 12.9 μmol/30 min, N = 6) or intraduodenal metformin (41.3 ± 14.7 μmol/30 min, N = 6).

GLUCOSE UPTAKE IN THE METFORMIN-TREATED RATS. Glucose uptake by the perfused intestine in the metformin-treated rats (45.1 ± 16.9 μmol/30 min, N = 6) was not significantly different from that in the control (sham-treated) rats (38.0 ± 13.3 μmol/30 min, N = 6).

Glucose Absorption by the Perfused Intestine

EFFECT OF INTRAVASCULAR OR INTRADUODENAL METFORMIN ON GLUCOSE ABSORPTION. As shown in Fig. 2, glucose absorption by the perfused intestine (788.1 ± 81.9 μmol/30 min) was not changed significantly by the addition of intravascular metformin (737.0 ± 118.2 μmol/30 min) or by intraduodenal metformin (772.8 ± 106.3 μmol/30 min).

GLUCOSE ABSORPTION IN THE METFORMIN-TREATED RATS. Glucose absorption by the perfused intestine in the metformin-treated rats (375.0 ± 164.3 μmol/30 min) was significantly ($P < 0.001$) lower than that in the control (sham-treated) rats (811.0 ± 83.1 μmol/30 min) (Fig. 3).

DISCUSSION

In the present study, the glucose absorption by the perfused intestine was not changed significantly by the direct addition of metformin to the perfusing medium or by intraduodenal metformin infusion, suggesting that metformin has no direct effect on glucose absorption in the intestines of rats. Wilcock and Bailey [12] have reported that the greatest accumulation of metformin occurs in tissues of the small intestine after oral administration, suggesting that the digestive tract is an important site for metformin treatment. Pénicaud *et al.* [20] and Bailey *et al.* [21] have suggested that metformin increases glucose utilization by the intestine in rats *in vivo*, and this may contribute to the blood glucose-lowering effect of the drug. In the present study, however, glucose uptake by the perfused intestine was not increased by the addition of intravascular or intraduodenal metformin, and intestinal glucose uptake was not increased in the metformin-treated rats. In their *in vivo* studies [20, 21], significant increases in glucose utilization by the digestive tract were observed in a hyperglycemic clamp study, and in the basal state a slight increase in glucose utilization was observed only in the jejunum [20].

An important observation in the present study is that glucose absorption by the perfused intestine was decreased significantly in the metformin-treated rats. Caspary and Creutzfeldt [13], Lorch [14], and Wilcock and Bailey [15] demonstrated that, in intestine rings and everted sacs of intestine from hamsters, rats, and mice, metformin decreases glucose transport by the intestine. Our present results were consistent with their studies. The precise mechanisms by which metformin administration decreased glucose absorption by the perfused intestine are unknown in the present study. Food consumption may affect glucose absorption from the intestine. However, daily food ingestion was not significantly different between control (13.5 ± 2.3 g/100 g body weight) and metformin-treated rats (12.9 ± 2.2 g/100 g body weight). The intestinal mucosa and/or enterocytes may have been somewhat damaged, because gastrointestinal symptoms are observed frequently in subjects treated with metformin. In the present study, however, histological examination did not show any changes in the intestinal mucosae of metformin-treated rats.

Lenzen *et al.* [22] reported that metformin treatment significantly increases energy-dependent sodium–hexose cotransporter (SGLT1) gene expression in the duodenum and jejunum, and facilitative hexose transporter GLUT5

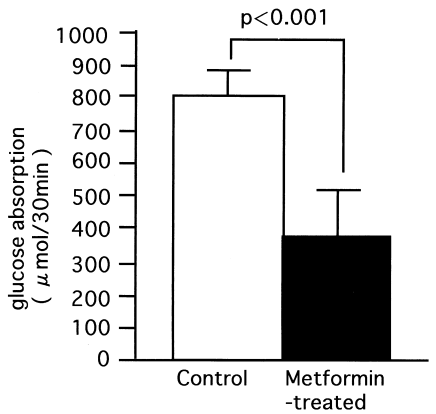

FIG. 3. Glucose absorption by the perfused intestine in metformin-treated rats. The bars represent means ± SD. The control group (N = 6) consisted of sham-treated rats. In the metformin group (N = 6), rats were treated with metformin (250 mg/kg body weight in 0.9% NaCl solution) by gastric catheter once daily for 5 days.

gene expression is increased in the jejunum. This offers the potential for increase in hexose uptake at the brush border membrane. Increased intestinal glucose utilization during metformin treatment would be expected to consume a greater proportion of the intracellular glucose taken up from the lumen, which accounts, at least in part, for the apparent reduction in glucose transport. This may explain the present phenomenon.

Although glucose absorption from the perfused intestine was decreased significantly, the blood glucose level was not changed significantly in metformin-treated rats. Further studies are necessary to determine whether the present phenomenon has a physiological significance in diabetic animals.

In summary, we conclude that metformin has a significant effect on the digestive tract, and metformin treatment exerts an inhibitory effect on glucose absorption by the perfused intestine of the rat.

References

1. Bailey CJ, Metformin revisited: Its actions and indications for use. *Diabet Med* **5:** 315–320, 1988.
2. Schafer G, Biguanides: A review of history, pharmacodynamics and therapy. *Diabete Metab* **9:** 148–163, 1983.
3. Jackson RA, Hawa MI, Jaspan JB, Sim BM, DiSilvio L, Fetherbe D and Kurtz AB, Mechanism of metformin action in non-insulin dependent diabetes. *Diabetes* **36:** 632–640, 1987.
4. Defronzo RA, Barzilai N and Simonson DC, Mechanism of metformin action in obese and lean noninsulin-dependent diabetic subjects. *J Clin Endocrinol Metab* **73:** 1294–1301, 1991.
5. Johnson AB, Webster JM, Sum CF, Heseltine L, Argyraki M, Cooper BG and Taylor R, The impact of metformin therapy on hepatic glucose production and skeletal muscle glycogen synthase activity in overweight type II diabetic patients. *Metabolism* **42:** 1217–1222, 1993.
6. Stumvoll M, Nurjhan N, Perriello G, Dailey G and Gerich JE, Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N Engl J Med* **333:** 550–554, 1995.
7. Radziuk J, Zhang Z, Wiernsperger N and Pye S, Effects of metformin on lactate uptake and gluconeogenesis in the perfused rat liver. *Diabetes* **46:** 1406–1413, 1997.
8. Widen EIM, Eriksson JG and Groop LC, Metformin normalizes nonoxidative glucose metabolism in insulin-resistant normoglycemic first-degree relatives of patients with NIDDM. *Diabetes* **41:** 354–358, 1992.
9. Matthaei S, Reibold JP, Hamann A, Benecke H, Haring HU, Greten H and Klein HH, *In vivo* metformin treatment ameliorates insulin resistance: Evidence for potentiation of insulin-induced translocation and increased functional activity of glucose transporters in obese (fa/fa) Zucker rat adipocytes. *Endocrinology* **133:** 304–311, 1993.
10. Giugliano D, De Rosa N, Di Maro G, Marfella R, Acampora R, Buoninconti R and D'Onofrio F, Metformin improves glucose, lipid metabolism, and reduces blood pressure in hypertensive, obese women. *Diabetes Care* **16:** 1387–1390, 1993.
11. Galauska D, Nolte LA, Zierath JR and Wallberg-Henriksson H, Effect of metformin on insulin-stimulated glucose transport in isolated skeletal muscle obtained from patients with NIDDM. *Diabetologia* **37:** 826–832, 1994.
12. Wilcock C and Bailey CJ, Accumulation of metformin by tissues of the normal and diabetic mouse. *Xenobiotica* **24:** 49–57, 1994.
13. Caspary WF and Creutzfeldt W, Analysis of the inhibitory effect of biguanides on glucose absorption: Inhibition of sugar transport. *Diabetologia* **7:** 379–385, 1971.
14. Lorch E, Inhibition of intestinal absorption and improvement of oral glucose tolerance by biguanides in the normal and in the streptozotocin-diabetic rat. *Diabetologia* **7:** 195–203, 1971.
15. Wilcock C and Bailey CJ, Reconsideration of inhibitory effect of metformin on intestinal glucose absorption. *J Pharm Pharmacol* **43:** 120–121, 1990.
16. Ikeda T, Mokuda O, Kuno S, Tokumori Y, Tominaga M and Mashiba H, Enhanced intestinal insulinotropic effect in streptozotocin-diabetic rats. *Am J Physiol* **248:** E304–E308, 1985.
17. Ikeda T, Yoshida T, Honda M, Ito Y, Murakami I, Mokuda O, Tominaga M and Mashiba H, Effect of intestinal factors on extraction of insulin in perfused rat liver. *Am J Physiol* **253:** E603–E607, 1987.
18. Levin SR, Pehlevanian MZ, Lavee AE and Adachi RI, Secretion of an insulinotropic factor from isolated, perfused rat intestine. *Am J Physiol* **236:** E710–E720, 1979.
19. Huggett AStG and Nixon DA, Use of glucose oxidase, peroxidase and *o*-dianisidine in determination of blood and urinary glucose. *Lancet* **273 (**Part 2**):** 368–370, 1957.
20. Pénicaud L, Hitier Y, Ferré P and Girard J, Hypoglycaemic effect of metformin in genetically obese (*fa/fa*) rats results from an increased utilization of blood glucose by intestine. *Biochem J* **262:** 881–885, 1989.
21. Bailey CJ, Mynett KJ and Page T, Importance of the intestine as a site of metformin-stimulated glucose utilization. *Br J Pharmacol* **112:** 671–675, 1994.
22. Lenzen S, Lortz S and Tiedge M, Effects of metformin on SGLT1, GLUT2, and GLUT5 hexose transporter gene expression in small intestine from rats. *Biochem Pharmacol* **51:** 893–896, 1996.