J. Pharm. Pharmacol. 1991, 43: 120–121
Communicated June 21, 1990

Reconsideration of inhibitory effect of metformin on intestinal glucose absorption

CAROL WILCOCK, CLIFFORD J. BAILEY, *Department of Pharmaceutical Sciences, Aston University, Birmingham B4 7ET, UK*

Abstract—New evidence that metformin increases intestinal glucose metabolism has necessitated a re-examination of the effect of metformin on intestinal glucose absorption. Normal 18 h fasted mice received an intragastric bolus of metformin 2 h before preparation of everted gut sacs from the proximal, middle and distal regions of the jejunum and ileum. Net mucosal glucose transfer from the intestinal lumen into the tissue was reduced by 15 and 28% after 50 and 250 mg kg^{-1} metformin, respectively (ANOVA, $P<0.05$). Net glucose transfer into the serosal fluid was reduced by 12 and 70% after 50 and 250 mg kg^{-1} metformin respectively (ANOVA, $P<0.05$ and $P<0.01$). The inhibitory effect of metformin on both the mucosal and serosal glucose transfer mechanisms was greatest in the middle portion of the small intestine. The results suggest that metformin decreases intestinal glucose absorption in a dose-dependent manner by effects on mucosal and serosal glucose transfer.

The glucose-lowering effect of metformin (dimethylbiguanide) has been attributed to increased peripheral glucose disposal and decreased hepatic glucose output (Bailey & Nattrass 1988). It has also been suggested that metformin might suppress intestinal glucose absorption, since the drug decreased glucose uptake by intestinal rings (Caspary & Creutzfeldt 1971) and decreased the appearance of glucose on the serosal side of everted gut sacs (Lorch 1971). However, the interpretation of these results must be re-examined in the light of recent reports that metformin increases intestinal glucose utilization (Bailey et al 1989a; Penicaud et al 1989; Wilcock & Bailey 1990), which could itself reduce the amount of glucose appearing at the serosal surface. Thus the present study was undertaken to determine simultaneously the effect of metformin on glucose uptake from the lumen and its appearance at the serosal surface of the intestine.

Materials and methods

Adult male albino MF1 mice, 25–35 g, were housed and maintained as described previously (Bailey et al 1989b). Metformin hydrochloride (batch 2452) was obtained from Lipha Pharmaceuticals Ltd, West Drayton, UK and all other chemicals were from BDH, Poole, UK. Groups of 18 h fasted mice received either metformin (50 or 250 mg kg^{-1}) or water (5 mL kg^{-1}) by gavage. After 2 h the mice were killed by cervical dislocation and the intestine from the ligament of Treitz to the ileocaecal junction was immediately removed and everted (Wilson & Wiseman 1954). The everted intestine was divided into 6 sacs corresponding to the proximal, middle and distal regions of the jejunum and ileum. Krebs Ringer bicarbonate (KRB) buffer (0·2 mL), pre-gassed with 95% O_2–5% CO_2, pH 7·4, supplemented with 5 mM glucose was injected into each sac. The sacs were weighed and incubated for 1 h at 37°C in 5 mL pre-gassed KRB buffer supplemented with 15 mM glucose. After incubation, sacs were blotted, weighed, emptied of their fluid content and re-weighed. The glucose concentration was determined (Stevens 1971) in the sac fluid (serosal fluid) and in the incubation medium (mucosal fluid). Changes in weight and glucose concentration were used to calculate net fluid and glucose movements (Levin 1967). Net mucosal glucose transfer (MGT) is the amount of glucose disappearing from the mucosal fluid, and net serosal glucose transfer (SGT) is the amount of glucose appearing in the serosal fluid. Glucose transfer values were expressed as μmol g^{-1} initial wet weight of tissue over the 1 h incubation period. Net mucosal fluid transfer (MFT) is the decrease in volume of mucosal fluid and net serosal fluid transfer (SFT) is the increase in volume of serosal fluid, expressed as μL g^{-1} wet weight of tissue.

Data were analysed by two-way analysis of variance (ANOVA) and individual groups were compared using Student's unpaired t-test. Differences were considered to be significant for $P<0.05$.

Correspondence to: C. J. Bailey, Department of Pharmaceutical Sciences, Aston University, Aston Triangle, Birmingham B4 7ET, UK.

Results and discussion

In control mice, MGT and SGT were affected by the region of small intestine (ANOVA, $P<0.05$), being greatest in the middle portion of the small intestine as shown in Fig. 1 and as reported by Newey (1967) and Pritchard & Porteous (1977). Intragastric administration of metformin (50 and 250 mg kg^{-1}) 2 h before incubation of tissue reduced MGT and SGT in a dose-dependent manner ($P<0.05$). The mean total MGT (proximal jejunum to distal ileum) was reduced by 15% ($P<0.05$) and 28% ($P<0.05$), and the mean total SGT was reduced by 12% ($P<0.05$) and 70% ($P<0.01$) after 50 and 250 mg kg^{-1} metformin, respectively. The greatest reduction occurred in the middle portion of the small intestine. MFT and SFT were reduced ($P<0.05$) by 250 mg kg^{-1} metformin (Fig. 2). The effect was most evident in the middle portion of the small intestine. The volume of fluid accumulated by the intestinal tissue (increase in wet weight of tissue) was not significantly altered by metformin (data not shown).

The present study provides evidence that metformin decreases both net glucose transfer from the lumen into the intestinal tissue

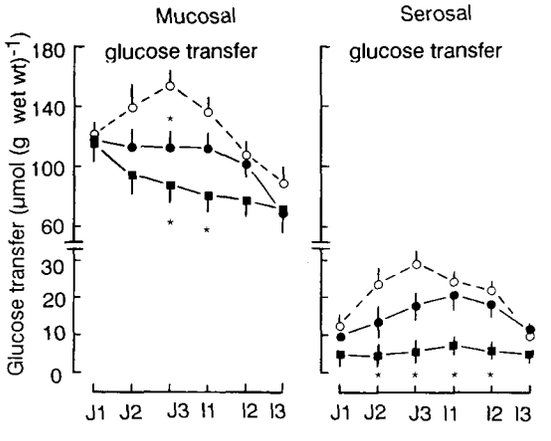

FIG. 1. Effect of metformin on mucosal glucose transfer and serosal glucose transfer by everted sacs of small intestine from mice. ○ Control; ● metformin 50 mg kg^{-1}; ■ metformin 250 mg kg^{-1}. Metformin was administered by gavage 2 h before preparation of everted sacs. Sacs were incubated for 1 h. J1, J2, J3, I1, I2, I3 refer to sacs prepared from the proximal, middle and distal regions of the jejunum and ileum. Values are mean ± s.e.m. of 6–10 determinations. *$P<0.05$ compared with control.

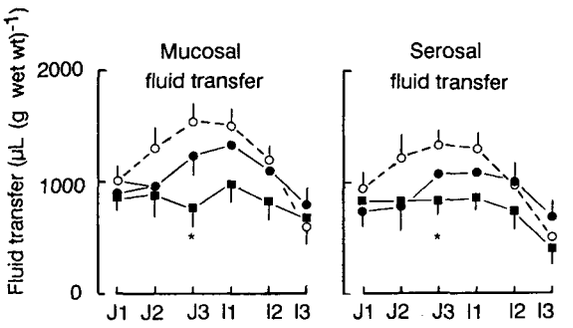

FIG. 2. Effect of metformin on mucosal fluid transfer and serosal fluid transfer by everted sacs of small intestine from mice. ○ Control; ● metformin 50 mg kg^{-1}; ■ metformin 250 mg kg^{-1}. Metformin was administered by gavage 2 h before preparation of everted sacs. Sacs were incubated for 1 h. J1, J2, J3, I1, I2, I3 refer to sacs prepared from the proximal, middle and distal regions of the jejunum and ileum. Values are mean ± s.e.m. of 6–10 determinations. *$P < 0.05$ compared with control.

(MGT) and net glucose transfer across the serosal boundary (SGT). Studies with isolated enterocytes have established that glucose transport at the mucosal brush border is secondary active and Na^+-dependent (Kimmich 1981; Hopfer 1987). Metformin might reduce MGT by a direct effect on the glucose transporter or indirectly by an unknown mechanism.

The reduction of SGT by 70% after an intragastric dose of 250 mg kg^{-1} metformin was comparable with a previous study (Lorch 1971). However, it is noteworthy that a therapeutically more relevant dose of 50 mg kg^{-1} metformin produced only a small (12%) reduction of SGT. Glucose transfer at the basolateral boundary of the enterocyte is mainly via a facilitated process which is Na^+-independent, the net flux being directed by the glucose concentration gradient across this boundary (Kimmich 1981; Hopfer 1987).

Reduction of SGT by metformin could arise by a number of mechanisms. Firstly, inhibition of MGT would be expected to reduce the amount of glucose available for transfer out of the tissue at the serosal boundary. In the present study, inhibition of SGT may not be entirely a consequence of the reduction of MGT, since the higher dose of metformin (250 mg kg^{-1}) reduced SGT for the whole intestine (70%) to a much greater extent than it reduced MGT (28%). Thus, in addition to an effect on the mucosal brush border, metformin appears able to affect glucose transfer at a subsequent step.

Secondly, metformin might increase the amount of glucose retained within the intestinal wall. This is difficult to quantify since glucose is rapidly metabolized by intestinal tissue. However, metformin did not increase fluid accumulation by the intestinal tissue, suggesting that the fluid for glucose distribution was not significantly altered. It is noteworthy that metformin reduced both MFT and SFT, which may reflect reduced solute transfer at both the mucosal and serosal boundaries (Parsons 1967).

Thirdly, the direction of net glucose transfer across the serosal boundary would be sensitive to changes in glucose utilization by the tissue. Recent studies in our laboratory using intestinal rings and in-vivo techniques have demonstrated that metformin increases glucose utilization by the intestine, associated with increased lactate production (Bailey et al 1989a; Wilcock & Bailey 1990).

Finally, metformin might act directly or indirectly to alter the number or activity of the transporters responsible for glucose transfer at the serosal boundary. Acute changes of glucose metabolism and lactate production by the small intestine are insulin-sensitive (Kellett et al 1984). Metformin can increase insulin receptor binding, glucose uptake and glucose metabolism in other insulin-sensitive tissues (Bailey 1988; Bailey & Nattrass 1988), and insulin receptors have recently been noted in the basolateral membrane of enterocytes (Gingerich et al 1987). Since SGT is a net measure of bidirectional glucose transfer, the relative contribution of unidirectional glucose movements cannot be distinguished by the present technique.

The present study has established that metformin reduced glucose uptake from the intestinal lumen as well as the transfer of glucose out of the tissue at the serosal boundary. Intestinal effects are envisaged to contribute together with increased peripheral glucose disposal and reduced hepatic gluconeogenesis to account for the antihyperglycaemic effect of metformin after an oral glucose challenge.

The authors gratefully acknowledge the helpful comments of Dr G. L. Kellet (University of York) and Dr M. S. Billingham (North Birmingham Hospital).

References

Bailey, C. J. (1988) Metformin revisited: its actions and indications for use. Diabetic Med. 5: 315–320

Bailey, C. J., Nattrass, M. (1988) Treatment-metformin. In: Nattrass, M., Hale, P. J. (eds) Clinical Endocrinology and Metabolism vol 2. Balliere Tindall, London, pp 455–476

Bailey, C. J., Wilcock, C., Bull, H., Day, C. (1989a) Metformin increases intestinal glucose utilization. Diabetic Med. 6 (Suppl. 2): 32A

Bailey, C. J., Conlon, J. M., Flatt, P. R. (1989b) Effect of ovarian hormones, pregnancy and lactation on somatostatin and substance P in the alimentary tract of mice. J. Endocrinol. 122: 645–650

Caspary, W. F., Creutzfeldt, W. (1971) Analysis of the inhibitory effect of biguanides on glucose absorption: inhibition of sugar transport. Diabetologia 7: 379–385

Gingerich, R. L., Gilbert, W. R., Comens, P. G., Gavin, J. R. (1987) Identification and characterization of insulin receptors in basolateral membranes of dog intestinal mucosa. Diabetes 36: 1124–1129

Hopfer, U. (1987) Membrane transport mechanisms for hexoses and amino acids in the small intestine. In: Johnson, L.R. (ed.) Physiology of the Gastrointestinal Tract (2nd edn). Raven Press, New York, pp 1499–1526

Kellett, G. L., Jamal, A., Robertson, J. P., Wollen, N. (1984) The acute regulation of glucose absorption, transport and metabolism in rat small intestine by insulin in vivo. Biochem. J. 219: 1027–1035

Kimmich, G. A. (1981) Intestinal absorption of sugar. In: Johnson, L. R. (ed.) Physiology of the Gastrointestinal Tract. Raven Press, New York, pp 1035–1061

Levin, R. J. (1967) Techniques, terminology and parameters in intestinal absorption. Br. Med. Bull. 23: 209–212

Lorch, E. (1971) 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

Newey, H. (1967) Absorption of carbohydrates. Br. Med. Bull. 23: 236–240

Parsons, D. S. (1967) Salt and water absorption by the intestinal tract. Ibid. 23: 252–257

Penicaud, L., Hitier, Y., Ferre, P., Girard, J. (1989) 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

Pritchard, P. J., Porteous, J. W. (1977) Steady-state metabolism and transport of D-glucose by rat small intestine in vitro. Ibid. 164: 1–14

Stevens, J. F. (1971) Determination of glucose by an automatic analyser. Clin. Chim. Acta 32: 199–201

Wilcock, C., Bailey, C. J. (1990) Sites of metformin stimulated glucose metabolism. Biochem. Pharmacol. 39: 1831–1834

Wilson, T. H., Wiseman, G. (1954) Metabolic activity of the small intestine of the rat and golden hamster (Mesocricetus auratus). J. Physiol. 123: 126–130