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Effects of Metformin on SGLT1, GLUT2, and GLUT5 Hexose Transporter Gene Expression in Small Intestine from Rats

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ABSTRACT. The effect of the antihyperglycaemic agent metformin was studied on gene expression of the energy-dependent sodium-hexose cotransporter (SGLT1) and the facilitative hexose transporters GLUT2 and GLUT5 in rat intestine. Metformin treatment (125 mg/kg body wt. twice daily for a period of 3 days) significantly increased SGLT1 gene expression in duodenum and jejunum. GLUT5 gene expression was increased by metformin treatment only in the jejunum. Gene expression of GLUT2 in the intestine was not significantly affected by metformin treatment. This increase in transporter gene expression offers the potential for increases in hexose uptake at the brush border membrane, and may compensate for other effects of the drug that have been suggested to decrease glucose uptake by SGLT1, as well as for metformin stimulation of glucose utilization by the intestinal mucosa. BIOCHEM PHARMACOL 51;7:893–896, 1996.

KEY WORDS. small intestine; SGLT1; GLUT2; GLUT5; gene expression; metformin

Metformin (dimethylbiguanide) is an antihyperglycaemic drug used in the treatment of noninsulin-dependent diabetes [1]. It reduces hepatic glucose output and increases glucose utilization [2]. In muscle, metformin enhances insulin-stimulated glucose uptake and oxidation without significantly increasing lactate production [3]. This has been attributed to increased translocation of the GLUT1+ transporter, and possibly also GLUT4, into the plasma membrane [4–6].

Recent studies have shown that metformin increases glucose utilization by the intestinal mucosa, and this may contribute to the blood glucose-lowering effect of the drug [7–9]. Unlike the effect on muscle, metformin causes a small suppression of glucose oxidation by intestinal mucosa while increasing lactate production. Because the intestinal mucosa accumulates a much higher concentration of metformin than muscle, it is possible that the drug acts through a different mechanism in the two tissues. Moreover, the intestinal mucosa does not express significant amounts of GLUT1 and GLUT4. In the intestinal mucosa, glucose and galactose are taken up at the brush border of enterocytes through the energy-dependent sodium hexose cotransporter SGLT1 [10–13]. Two further hexose transporter isoforms are expressed by enterocytes. Fructose is taken up at the brush border through the facilitative hexose transporter GLUT5 [10, 11, 14–17]; glucose, galactose, and fructose are exported from the enterocyte across the basolateral membrane through the facilitative hexose transporter GLUT2 [10, 11, 15, 17, 18].

To investigate the mechanism through which metformin affects the intestinal handling of hexoses, the present study examines the effect of metformin on the gene expression of SGLT1, GLUT2, and GLUT5 in rat intestine.

MATERIALS AND METHODS

Materials

The SP6/T7 Transcription Kit, DIG Nucleic Acid Detection Kit, and restriction enzymes were from Boehringer, Mannheim, Germany and Hybond N hybridization transfer membranes from Amersham, Braunschweig, Germany. Guanidine thiocyanate was purchased from Fluka, Neu-Ulm, Germany. Metformin hydrochloride was kindly provided by Lipha, Essen, Germany. All other reagents of analytical grade were from Merck, Darmstadt, Germany.

Animals and Tissue Preparation

Male Wistar rats (290–340 g) were fed a standard laboratory diet containing 50% complex carbohydrate (Altromin, Lippe, Germany). The animals were housed in individual cages at a constant temperature of 22°C in a light-cycled animal care facility with free access to the diet and water. Metformin (125 mg/kg body wt. in 0.9% NaCl solution) was administered through a gastric catheter after light ether anesthesia twice daily for a period of 3 days. The controls received 0.9% NaCl solution. Normal food consumption was maintained during the metformin treatment, and ex-

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† *Abbreviations:* SGLT1, sodium-dependent glucose transporter type 1; GLUT2, facilitative glucose transporter type 2; GLUT5, facilitative glucose transporter type 5.

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periments were performed in the fed state. Rats were killed 2 hr after the last metformin administration by decapitation under ether anesthesia and the organs removed immediately. The small intestine from the pylorus to the cecum was removed and perfused with 120 mL ice-cold 1.32% NaCl solution. Six segments (1–6) 3 cm in length were taken at a distance of 5, 10, 30, 50, 70, and 90 cm, respectively, from the pylorus. Segment 1 corresponds to the duodenum, 2 to the proximal jejunum, 3 and 4 to the midjejunum, 5 to the distal jejunum, and 6 to the ileum.

Northern Blot Analysis

Total RNA from small intestine segments was isolated by a combined water-saturated phenol-chloroform-isoamyl alcohol extraction according to Chomczynski and Sacchi [19]. RNA, 20 μg total RNA, 20 μg per lane, was subjected to electrophoresis on denaturing formamide/formaldehyde 1% agarose gels and blotted to nylon membranes. Under conditions described previously [20], nonradioactive hybridization was performed using 11-DIG-UTP-labeled antisense cRNA probes coding for the rabbit SGLT1 hexose transporter [21], the rat GLUT2 hexose transporter [18], and the rat GLUT5 hexose transporter [16]. The DIG-labeled hybrids were detected by an enzyme-coupled immunoassay using an antiDIG-alkaline-phosphatase antibody conjugate. The hybrids were visualized by chemiluminescence on a light-sensitive film. The autoradiograms were quantified by densitometry using the Image 1.52 analysis program (NIH, Bethesda, MD). Ribosomal bands were used as control markers for gel loading.

Determination of Metformin Serum Concentration

Metformin was determined by cation-exchange chromatography according to Lacroix *et al.* [22]. After deproteinization with TCA, 50 μL serum obtained through cardiac puncture was applied on a 5 μm Biokon KAT Pep column (Kontron, Neufahrn, Germany) at a flow rate of 1 mL/min with 0.1 M $(NH_4)H_2PO_4$ as mobile phase. Metformin was detected at 232 nm. The peaks were quantified by the Kontron Integration Pack program.

Statistics

Experimental data are expressed as mean values ± SEM. Statistical analyses were performed using the Student's *t*-test.

RESULTS

Distribution of Hexose Transporter Gene Expression

In the nontreated rat, gene expression of SGLT1 (4.5 kb), GLUT2 (2.8 kb), and GLUT5 (3.2 kb) was observed in all segments of intestine (Fig. 1). The level of expression was typically higher in the duodenum (segment 1) and proximal jejunum (segment 2), decreasing towards the ileum (segment 6) (Fig. 1). There was no gene expression of hexose transporters detectable in the colon (data not shown). Compared with segment 3 (midjejunum), gene expression in the ileum was reduced by 25%, 86%, and 50% for SGLT1, GLUT2, and GLUT5, respectively (Table 1). Thus, the relative expression of SGLT1 was maintained at higher levels in the ileum than the expression of GLUT2 or GLUT5.

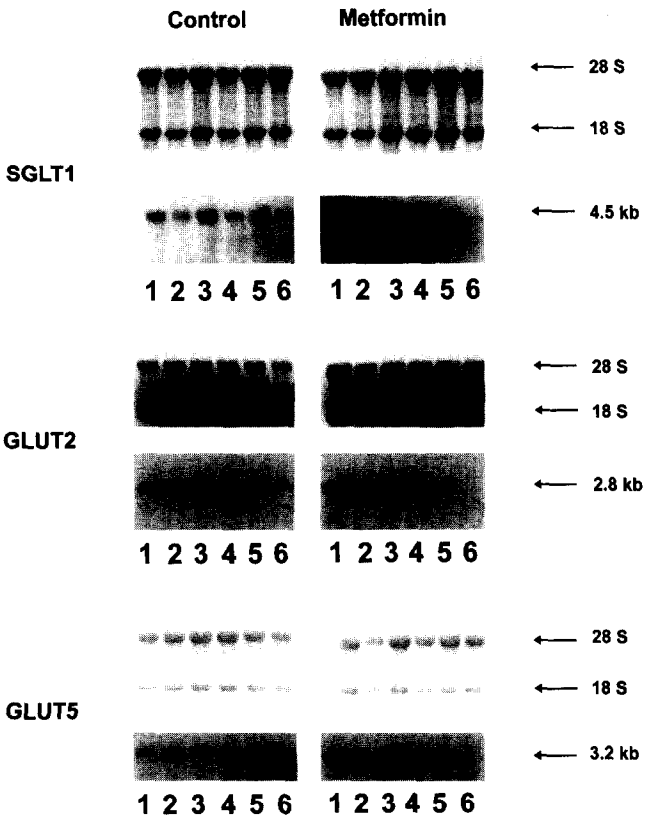

FIG. 1. Northern blot analysis of SGLT1, GLUT2, and GLUT5 hexose transporter gene expression in rat small intestine segments after metformin treatment (1, duodenum; 2, proximal jejunum; 3, 4, midjejunum; 5, distal jejunum; 6, ileum). Metformin (125 mg/kg body wt. in 0.9% NaCl solution) was administered to the animals twice daily for a period of 3 days. 20 μg total RNA from each intestinal segment was hybridized using antisense cRNA probes coding for SGLT1, GLUT2, and GLUT5 hexose transporter. Blots were related to the 28 S ribosomal bands for equal loading of the gel, as shown in the upper panel. Shown are representative blots of 5–9 independent experiments.

Effects of Metformin

After treatment with metformin (125 mg/kg twice daily for 3 days), daily food consumption was not altered. The serum concentration of metformin at the time of experimentation (2 hr after the last intragastric administration) was 4.49 ± 0.44 μg/mL (34.5 ± 3.4 μM) (n = 10). Metformin significantly increased SGLT1 gene expression in the duodenum and jejunum (Fig. 1 and Table 1). For the total intestine, overall SGLT1 gene expression after metformin treatment

TABLE 1. Effects of metformin treatment on SGLT1, GLUT2, and GLUT5 glucose transporter gene expression in small intestine from rats

	SGLT1 ($n = 6$)		GLUT2 ($n = 9$)		GLUT5 ($n = 5$)	
Intestinal segment	**Control**	**Metformin**	**Control**	**Metformin**	**Control**	**Metformin**
1 (duodenum)	143 ± 26	349 ± 57*	106 ± 10	136 ± 37	217 ± 92	212 ± 52
2 (proximal jejunum)	157 ± 69	325 ± 48	97 ± 11	105 ± 12	131 ± 20	216 ± 41
3 (midjejunum)	100 ± 13	372 ± 81*	100 ± 12	126 ± 18	100 ± 4	189 ± 34*
4 (midjejunum)	97 ± 25	254 ± 43*	84 ± 9	92 ± 14	83 ± 13	137 ± 10*
5 (distal jejunum)	70 ± 22	71 ± 24	52 ± 16	22 ± 6	97 ± 17	94 ± 36
6 (ileum)	75 ± 29	84 ± 40	14 ± 7	1 ± 1	50 ± 22	40 ± 23
Overall intestine†	107 ± 28	243 ± 49*	75 ± 9	81 ± 15	113 ± 27	148 ± 33

Metformin (125 mg/kg body wt. in 0.9% NaCl solution) was administered to the animals twice daily for a period of 3 days. 20 μg total RNA from each intestinal segment was hybridized using antisense cRNA probes coding for SGLT1, GLUT2, and GLUT5 hexose transporters. Gel loading was corrected to the ethidium-bromide stained 28 S ribosomal bands by densitometric quantification of polaroid films. The gene expression of segment 3 from control rats was set at 100%. Values are presented as means ± SEM for the number of experiments indicated. *$P < 0.05$ compared with control; †Overall intestine is the mean ± SEM of all segments examined.

was 121% greater than the control (Table 1). GLUT5 gene expression was increased by metformin treatment only in the jejunum (Fig. 1 and Table 1). In contrast, gene expression of the GLUT2 transporter was not significantly altered by metformin treatment. The pattern of hexose transporter gene expression in the different segments of intestine was not affected by metformin (Table 1 and Fig. 1).

DISCUSSION

The present study demonstrates a greater expression of the SGLT1 gene towards the proximal end of the intestine, although there is still substantial expression of the gene through to the ileum. This is compatible with an increased capacity for Na^+-glucose cotransport across the brush border membrane of enterocytes in the more proximal regions of the small intestine [23]. Taking into account the more extensive villi of the jejunum, the present data are also consistent with the greater absorptive function of this portion of the intestine [24]. Gene expression of the fructose transporter GLUT5 showed a similar distribution to that of SGLT1, although GLUT5 expression was less well maintained in the ileum. Expression of the GLUT2 gene declined considerably in the ileum, as might be expected in a region where sugar absorption is reduced.

Administration of metformin (125 mg/kg twice daily for 3 days) produced a serum concentration in the range of 5–6 μg/mL 2 hr after the last administration, only slightly higher than the typical peak concentration of 1–3 μg/mL (approximately 10^{-5} M) observed during normal therapeutic use of this drug [1]. Although metformin concentration in muscle is similar to serum, the drug is accumulated by intestinal mucosa to approximately 10^{-3} M [25]. Early studies using intestine rings and everted sacs of intestine from hamsters and rats suggested that concentrations of metformin > 10^{-3} M could decrease glucose transport by the intestine [26, 27]. However, these studies did not take into account its more recently discovered effect of increasing glucose utilization by the intestine [7, 8].

The present observation of increased SGLT1 gene expression during metformin treatment cannot be explained by alteration of food intake, but this drug could increase the capacity of enterocytes to undertake Na^+-glucose cotransport across the brush border. However, whether or not this capability is accomplished in practice is uncertain. Indeed, a study with brush border vesicles from rabbit intestine has noted that 5×10^{-3} M metformin slightly decreased glucose uptake [28]. This was attributed to the high concentration of drug providing extra positive charges in the membrane and the consequent reduction in the sodium concentration of the local membrane microenvironment, thereby reducing occupancy of SGLT1. Moreover, 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 noted in early studies [26, 27].

Increased expression of GLUT5 in the jejunum of metformin-treated rats suggests an increased capability of fructose uptake from the intestinal lumen, although the effect of metformin on fructose handling by the intestine has not yet been examined.

Metformin treatment did not alter GLUT2 gene expression by enterocytes. However, the high capacity of this facilitative transporter to transfer hexoses across the basolateral membrane would be sufficient to accommodate large changes in the rate of glucose entry across the brush border. Because metformin can increase 2-deoxy-D-glucose uptake by the intestinal mucosa [9], which is not transported by SGLT1, this suggests that the drug can increase glucose uptake by GLUT2. This could occur during fasting when the concentration of glucose within the enterocyte is reduced. Such an effect cannot be attributed to an alteration of GLUT2 expression.

The present study demonstrates that metformin treatment increases the gene expression of SGLT1 and GLUT5 in rat jejunal enterocytes. This offers the potential for increases in hexose uptake at the brush border membrane, and may compensate for other effects of the drug that have been suggested to decrease glucose uptake by SGLT1 as

well as for metformin stimulation of glucose utilization by the intestinal mucosa. Thus, during clinical use of the drug, there may be a slight delay in the transfer of glucose from the gut lumen into the circulation, but the uptake of a glucose load is complete [29].

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