Y. SAKAR[1], B. MEDDAH[2], MY.A. FAOUZI[2], Y. CHERRAH[2], A. BADO[1], R. DUCROC[1]

METFORMIN-INDUCED REGULATION OF THE INTESTINAL D-GLUCOSE TRANSPORTERS

[1]Institut National de la Sante et de la Recherche Medicale U773, Centre de Recherche Biomedicale Bichat-Beaujon, CRB3, Paris, France; Universite Denis Diderot, UMR S 773, Paris, France; [2]Equipe de Recherche de Pharmacocinetique, Laboratoire de Pharmacologie et Toxicologie, Faculte de Medecine et de Pharmacie, B.P. 6203 Rabat-Instituts, Rabat, Morocco.

Metformin is an orally administered drug that lowers blood glucose and improves insulin sensitivity in patients with non insulin-dependent diabetes. Although the antihyperglycemic effect of metformin has been extensively studied, its cellular mechanism(s) of action (including the effect on enterocyte) remains to be defined. This study was designed to examine the effect of metformin on glucose transporters in enterocyte. Na^+-dependent glucose transporter-1 (SGLT-1) activity was followed as glucose-induced short-circuit current (Isc) in Ussing chambers. The effect of metformin (10 μmol/L, 3 min) on transmural glucose transport was studied in isolated rat jejunal loops. Its impact on abundance of transporters SGLT-1 and GLUT2 in jejunal brush border membranes (BBM) and its effect on the phosphorylation of AMP-activated protein kinase (AMPK) α2 subunit was studied by western blot. Acute effect of metformin was also measured *in vivo* by oral glucose tolerance test (OGTT). Metformin markedly inhibited glucose-induced Isc (~77%) after mucosal addition. In addition, metformin reduced the glucose-induced abundance of SGLT-1 in BBM and increased those of GLUT2, concomitantly increasing the phosphorylation of intracellular AMPKα2. This effect of metformin was also observed using non-metabolizable sugar α3-O-methyl glucose. Transmural glucose transport measured *in vitro* was increased by 22% under metformin. Finally, oral metformin markedly increased glucose tolerance in OGTT. In conclusion, metformin slightly increases intestinal glucose absorption by inducing a re-distribution of glucose transporters in BBM through AMPK control in enterocyte. In addition to its action to other splanchnic tissues, this could constitute a peripheral signal contributing to the beneficial effect of metformin on glucose tolerance.

K e y   w o r d s: *metformin, ussing chamber, glucose tolerance, AMP-activated protein kinase, α3-O-methyl glucose, sodium-dependent glucose transporters-1, diabetes*

INTRODUCTION

Dimethylbiguanide metformin or "metformin" is an orally administered drug that lowers the blood glucose concentration and improves the insulin sensitivity in type 2 diabetes. Metformin is one of the most prescribed generic drugs with ~35 millions doses per year that is given as monotherapy in early stages of type 2 diabetes or in combination with most of the currently available antidiabetic drugs (1). The mechanisms of action of metformin as antihyperglycemic agent have extensively been studied (2). Its beneficial effect on blood glucose appears to be a result of complex multifactorial mechanisms including i) decreased hepatic neoglucogenesis leading to reduced glucose output (3-5); ii) improved sensitivity to insulin; iii) increased utilization of glucose by the small intestine (5, 6) and iiii) increased uptake of glucose by the skeletal muscles and white adipocytes (7-9). Intraduodenal administration has been shown to be the major route for an optimum overall glucose-lowering response to metformin in human (10). After oral administration, metformin is absorbed by the proximal small intestine through an active, saturable process (1, 11, 12) mediated by a specific metformin pH-dependent transporter present at the apical membrane of human enterocytes (13).

Brush border membrane (BBM) of enterocyte is a primary site of dietary sugars' absorption. The Na^+/glucose cotransporter SGLT-1 transports glucose and galactose from the lumen of the intestine into enterocytes (14). SGLT-1 appears also to be a functional part of a peripheral system that senses exogenous glucose to maintain energy homeostasis (15). SGLT-1 activity is highly regulated by several peptides and hormones active at either mucosal (16-18) or serosal (19-21) enterocyte membranes. Regulation of SGLT-1 involves a post translational mechanism that allows rapid ajustments of protein abundance in the BBM and recruitment of the permease GLUT2 depending on luminal sugar levels (22) and hormones (23, 24). SGLT-1 and GLUT2 activities are increased in type 2 diabetes (25, 26), likely as a result of deregulation. Control of glucose absorption by modulation of the glucose transporters's activity appears to be a key mechanism and may be a paradigm for action of orally active drugs to maintain normal blood glucose.

This work was designed to study the effect of metformin on SGLT-1 and to examine the intracellular mechanisms of its action

on glucose transporters in enterocyte. We also studied the effect of metformin on transmural glucose transport and the consequences of this rapid effect on circulating blood glucose in conscious rat.

MATERIAL AND METHODS

Animals

Male Wistar rats weighing 240-280 g (Centre Elevage Janvier, Le Genest-St-Isle, France) were caged under standard laboratory conditions with tap water and regular food provided *ad libitum*, in a 12-h/12-h light/dark cycle at a temperature of 21-23°C. The animals were treated in accordance with the European Community guidelines based on declaration of Helsinki concerning the care and use of laboratory animals and all efforts were made to minimize animal suffering and the number of animals used.

Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed on conscious rats, following a 18 hours fasting. Blood samples from fasted animals were first taken from the tail vein by 10:00 o'clock in the morning. Metformin (1,1-dimethylbiguanide hydrochloride) from Sigma-Aldrich (Meylan, France) was diluted in NaCl 0.9% and given orally (300 mg/kg) 3 min before the load of glucose. Controls were given NaCl 0.9%. Rats in all groups were fed a 30% D-glucose solution (2 g/kg body weight) and blood samples taken by tail bleeds at 15, 30, 60, 90 and 120 min after glucose administration. Glucose determination in blood was run immediately using an Accu-Chek Go (Roche Diagnostics, Meylan, France).

Tissue preparation and short-circuit measurement

Rats were fasted 16 hours with water *ad libitum*. Animals were sacrificed by i.p. pentobarbital overdose and the proximal jejunum was dissected out and rinsed in cold saline solution. Four adjacent proximal samples were mounted in Ussing chambers as previously described (16, 27). The tissues were bathed on each side with carbogen-gassed Krebs-Ringer bicarbonate (KRB) solution having the following composition (in mmol/L): NaCl 115.4, KCl 5, $MgCl_2$ 1.2, NaH_2PO_4 0.6, $NaHCO_3$ 25, $CaCl_2$ 1.2 and glucose 10. In the solution bathing the mucosal side of the tissue, glucose was replaced with mannitol. Mannitol was kept in the bathing solution during glucose challenge. Both solutions were gassed with 95% O_2-5% CO_2 and kept at constant temperature of 37°C (pH at 7.4).

Electrogenic ion transport was monitored continuously as short-circuit current (Isc) using the Easy Mount System apparatus (Physiological Instruments, Inc., San Diego, USA) and a DVC 1000 voltage clamp (WPI, Aston, England). Metformin was added in the mucosal bath 3 min before luminal glucose challenge. Results were expressed as the difference (ΔIsc) between the peak Isc after glucose challenge (maximum measured after ~3 min) and the basal Isc (measured just before the addition of glucose).

Transepithelial transport of glucose in jejunal loop ex vivo

Sixteen-hour fasted rats were sacrificed, laparotomized and segments of jejunum (~5 cm) were removed and filled with 100 μL/cm of a Krebs modified buffer pH 6 containing 30 mM glucose and 50 mCi/mmol of [14C] glucose in absence (control) or presence of 10 μmol/L metformin in the lumen of the loop. Effect of metformin on paracellular permeability was evaluated

using [14C] mannitol. Each segment was incubated in a 37°C thermostat-controlled bath of Krebs modified buffer at pH 7.4 continuously gassed with 95% O_2-5% CO_2. In those experiments in which cytochalasin B or 6-[4-(2-piperidin-1-ylethoxy)phenyl)]-3-pyridin-4-ylpyrazolo[1,5-a] pyrimidine (compound C, Sigma Chemicals, St Louis Mo USA) were used, the segments were preincubated for 5 min before the addition of the solution for time course study in absence or presence of metformin, with the inhibitor also present in glucose solution.

Incubation medium were sampled at various time points from 0 to 60 min. The radioactivity counted in the collected samples was used to calculate amount of glucose. Apparent permeability (P_{app}) was used to assess mucosal to serosal glucose transport according to the following equation P_{app}= (dQ/dt).(V/Q_0.A) where V is the volume of the incubation medium, A is the area of the loop, Q_0 is the total radiolabelled 14C- glucose introduced into the loop and dQ/dt is the flux across the jejunum loop.

Western blot analysis

Fasted rats were anesthetized by i.p. administration of pentobarbital and laparotomized. Three jejunal loops (7 cm long) were prepared as previously described (10, 16, 22, 23) and filled with 3 mL of KRB-mannitol without (control) or with 10 μmol/L metformin. After 3 min *in vivo* incubation, the loops were injected with ~1 ml of a 10 mM-D glucose solution and again incubated for 3 min. After sacrifice, loops were removed, opened along the mesenteric border and the mucosa was scraped off on ice with a glass blade. In some experiments, 50 μmol/L compound C was injected into the intestinal loop and allowed to stay for 30 min before metformin administration. For total protein extraction, jejunal mucosa scrapings were homogenized at 4°C in lysis buffer (250 mM sucrose tris/HCL 50 mM, ph 7.35) supplemented with 0.1 mg/ml phenylmethylsulfonyl fluoride, 100 μmol/L aprotinin, and 100 mmol/L $NaVO_4$. The homogenates were centrifuged at 15000 g for 30 min at 4°C, and the supernatants were collected for western blot analysis of total cell proteins. BBM were prepared from jejunal mucosa scrapings as described (16, 23). Protein concentration was quantified using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). Solubilized proteins were resolved by electrophoresis on 12.5% SDS-PAGE gels. The resolved proteins were transferred onto nitrocellulose membranes and subjected to immunoblot analysis with a rabbit anti-SGLT-1 polyclonal antibody (AB 1352, Chemicon Millipore, Temecula, CA) diluted 1:7,500 and reprobed with anti-GLUT2 polyclonal antibody (sc-9117, Santa Cruz Biotechnology Inc., Santa Cruz CA) diluted 1:500 or with rabbit polyclonal P-AMPKα2 (sc-101631, Santa Cruz Biotechnology Inc., Santa Cruz CA) diluted 1:500 or with mouse monoclonal β-actin antibody or total-AMPK antibody (sc-81178 and sc-25792 respectively from Santa Cruz Biotechnology Inc., Santa Cruz CA). Immune complexes were detected by enhanced chemiluminescence (Pierce, Rockford, IL). The intensity of the immunoreactive bands was quantified using NIH Image (Scion Corp. Frederick Maryland, USA). β-actin or total AMPK were used as loading controls. The results were expressed in relation to control and the value of control was arbitrarily set to 1.

Statistical analysis

Results are expressed as means±SEM with n = number of tissues. One-way ANOVA with Tukey-Kramer multiple comparison post test was performed using GraphPad Prism version 3.0 for Windows (Graphpad software Inc., San Diego, CA). $p < 0.05$ was considered significant.

RESULTS

Luminal metformin inhibits Na^+-dependent glucose absorption

The effect of metformin on glucose absorption was studied *in vitro* on rat jejunum isolated in Ussing chamber (16, 23, 27). Luminal addition of 10 mmol/L D-glucose to mucosal bath of the Ussing chamber induced a rapid and marked increase in Isc (*vs.* basal conditions), which plateaued after 3 min (*Fig. 1A*). Mucosal addition of metformin 3 min before glucose challenge markedly reduced the glucose-induced Isc (ΔIsc; *Fig. 1B*). As depicted on *Fig. 1C*, inhibition of ΔIsc was dose-dependent with a maximal inhibition observed for a concentration of 1 μmol/L and a IC_{50} of 8 nmol/L.

Metformin inhibits SGLT-1 and increases GLUT2 abundance in BBM

We then examined whether metformin can modify glucose transporters abundance in BBM after a 3-min incubation time *in vivo*. As shown in *Fig. 2*, metformin (10 μmol/l) significantly reduced the basal level of SGLT-1 in BBM. Glucose (10 mmol/l) induced a 1.7-fold increase of SGLT-1 amounts in BBM compared to controls. This increase in SGLT-1 abundance was significantly prevented by the presence of 10 μmol/l metformin injected into the loop 3 min before glucose. No significant change in the amount of SGLT-1 immunoreactive protein in total protein extracts was noticed after the glucose challenge, neither in the presence nor in the absence of metformin (not shown).

When the same membranes were further studied with GLUT2 antibody, we observed a significant effect of metformin to increase abundance of GLUT2 in BBM. Glucose alone also induced a 2.1-fold increase in apical GLUT2 protein (lane 3). Metformin given before glucose further increased immunoreactive GLUT2 in BBM (*Fig. 2B*, lane 4).

As glucose utilization by enterocyte may constitute a signal for this regulation, same experiments were performed using non-metabolized substrate 3-O-methyl glucose (3-OMG). No differences were found in SGLT-1 (+2.2 fold increase *vs.* +1.7 fold) or GLUT2 (+2.2 fold increase *vs.* +1.9 fold) abundance in BBM with 3-OMG or glucose alone, respectively. Metformin reduced the sugar-induced abundance of SGLT-1 by 43 and 45% in 3-OMG and glucose, respectively and induced an increase of 21% of GLUT2 with both sugars.

Metformin increases phosphorylation of AMPK α2 in jejunal mucosa

We also examined if metformin alone or in combination with glucose was able to modify the activity of mucosal AMPK by measuring the abundance of phosphorylated immunoreactive form of α2 subunit. As shown, 10 μmol/L metformin significantly increased the level of phosphorylated α2 AMPK (2.9-fold). This effect was also observed in the presence of glucose alone (see above) where the effect of metformin was even more pronounced reaching 3.8-fold the control level (*Fig. 2C*).

AMPK phosphorylation in response to 3-OMG was not significantly modified when compared to glucose (+2.7 fold increase *vs.* +2.5 fold in glucose). In presence of metformin AMPK phosphorylation reached 3.5 and 3.3 fold, respectively (data not shown).

Compound C prevents the effect of metformin on glucose transporters

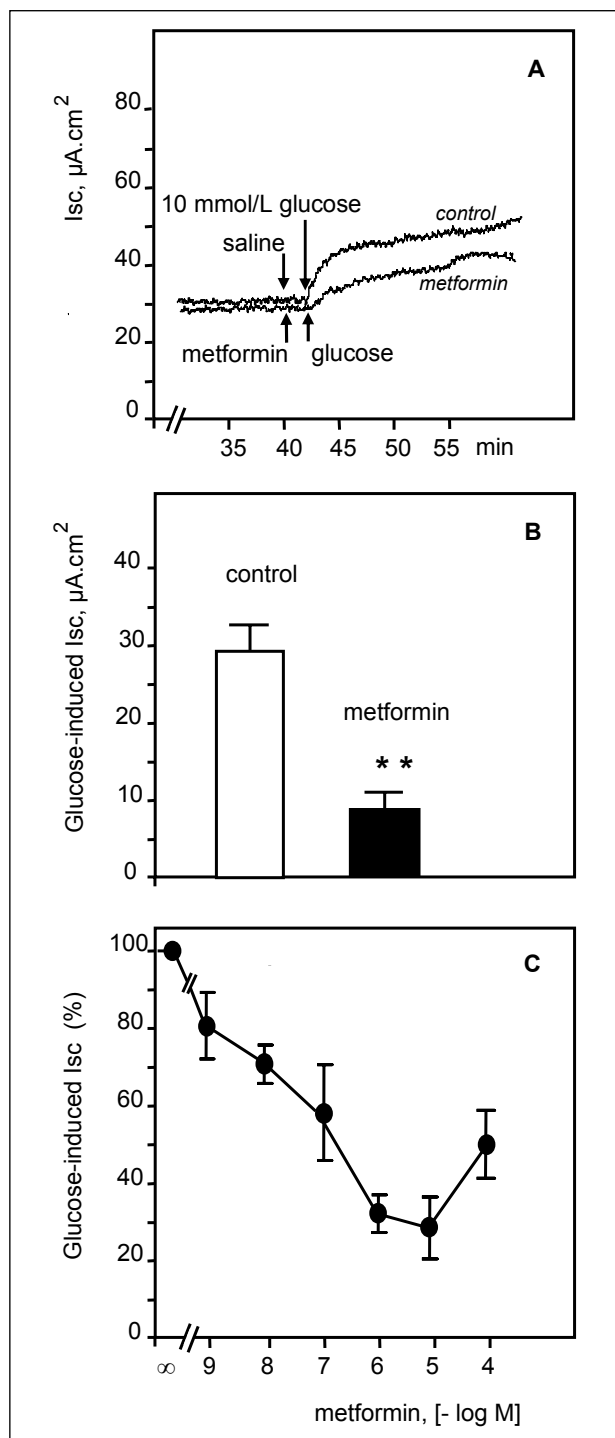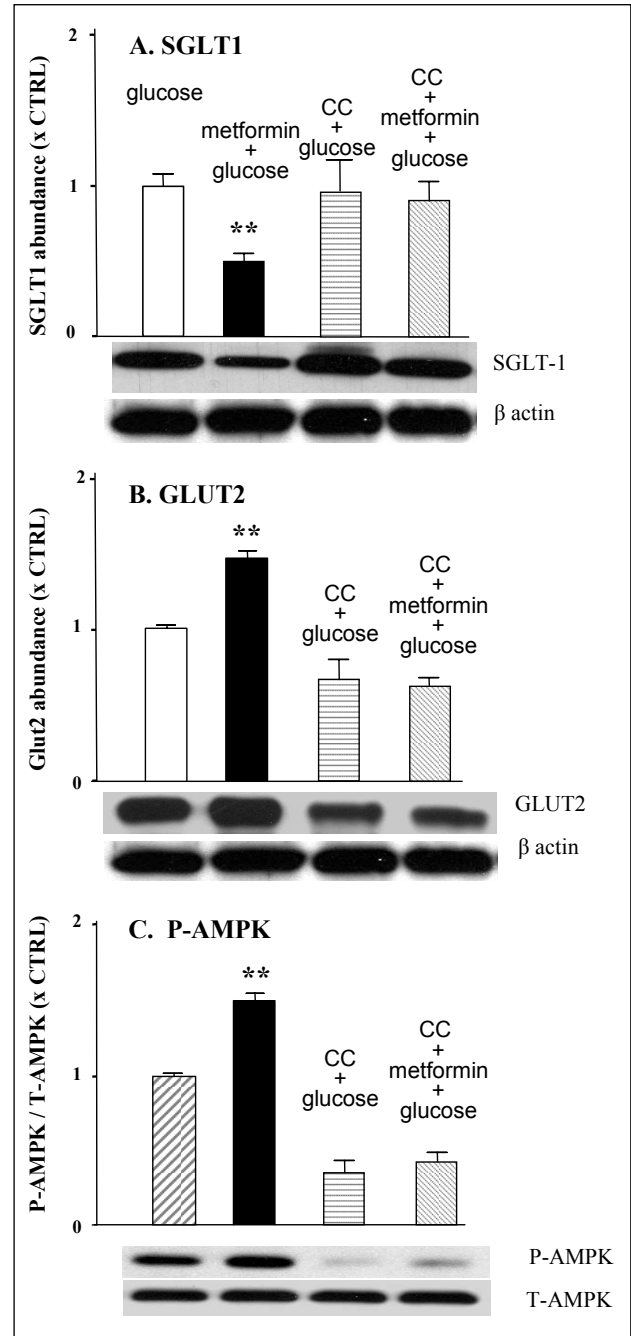We studied the effect of compound C, an inhibitor of AMPK phosphorylation, on metformin-induced regulation of

Fig. 1. Effect of luminal metformin on glucose-induced short-circuit current (Isc). Non-cumulative response curve to metformin was achieved by measuring ΔIsc response to a luminal 10 mmol/L-glucose challenge (see Material and Methods). (A) Typical recording of Isc in isolated rat jejunal tissus. Metformin (or saline) was added in luminal compartment of Ussing chamber 3 min before glucose challenge; (B) Effect of 1 μmol/L metformin on glucose-induced Isc (in μA.cm²). ** , p<0.01; (C). Response curve to metformin. Each point is mean±SEM of 4-6 tissues. Note that maximum inhibition is observed for 1 μmol/L metformin and IC_{50}= ~8 nmol/L.

glucose transporters. As expected (*Fig. 3B*), compound C prevented the metformin-induced phosphorylation of AMPKα. Luminal instillation of metformin in isolated loops of small
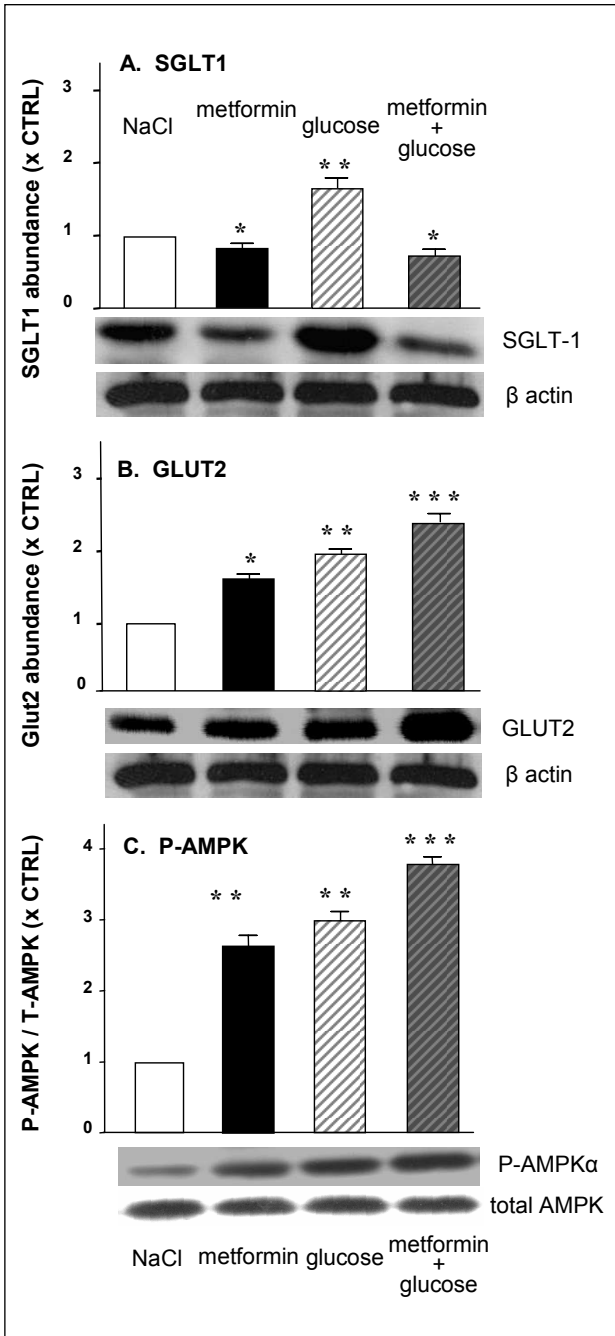
Fig. 2. Effect of metformin on expression of glucose transporters and phosphorylated form of AMPKinase α2 (p-AMPK α2) in jejunal mucosa. Sodium-dependent glucose transporter-1 SGLT-1 was analyzed by western blot in proteins prepared from the brush border membranes, then the blots were reprobed for GLUT2 and β-actin (typical image is shown). AMPK was studied in total protein from mucosal preparation. Data showed in the graph are mean±SEM of 3-5 determinations. Value of control (NaCl, 0.9% NaCl) was assessed to 100%. * , statistically significant from control, p<0.05; * * , p<0.01; * * *, p<0.001.

Fig. 3. Effect of Compound C (CC) on metformin-induced control of glucose transporters and p-AMPK. CC was injected in intestinal loops 30 min before metformin or saline (0.9% NaCl). After another 3 min-period, glucose was injected and loops were kept warm for another 3 min before dissection of loops and preparation of proteins (total and brush border proteins), as presented in *Fig. 2.* ** : statistically significant from glucose only, p<0.01.

intestine rapidly modulated immunoreactive SGLT-1 and GLUT2 abundance in BBM in opposite manner and this was totally blocked by compound C. These data suggest that the effect of metformin on the relative abundance of glucose transporters in apical membrane is mediated by AMPK activation.

Metformin modulates 14-C glucose transepithelial transport

Effect of metformin on transmural glucose transport was evaluated in isolated loops prepared from rat jejunum. Luminal glucose (30 mmol/L) instilled in the loop was followed as a net mucosal-to-serosal glucose flux using ^{14}C-glucose (14.8±1.32 pmol/mg prot/min). Luminal metformin enhanced the jejunal transport of glucose by 18% (p<0.05; *Fig. 3*) but had no effect on 30 mM mannitol transport (not shown). Thus, the increase in
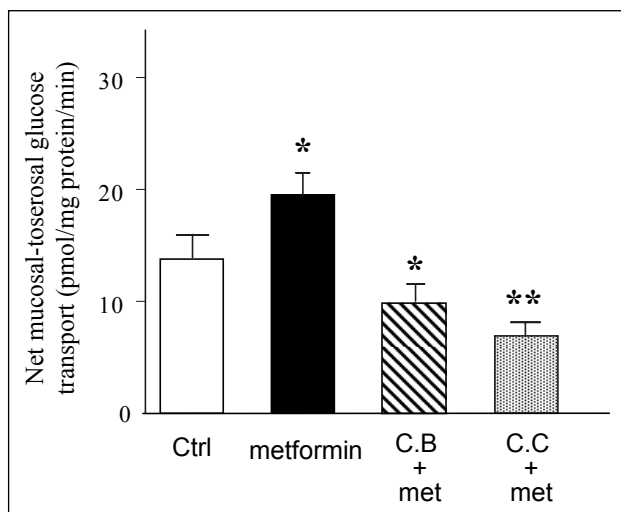
Fig. 4. Effect of metformin on transmural glucose transport *ex vivo*. Jejunal loops were prepared and incubated with a Krebs Ringer solution containing 30 mM glucose. Metformin or saline (0.9% NaCl) was added to the luminal side. In some experiments, loops were pretreated with cytochalasin B (CB) or compound C (CC) for 5 min. Transmural transport of glucose was studied as ^{14}C-glucose by liquid scintigraphie * , statistically significant compared to glucose only, p<0.05; **, p<0.01.
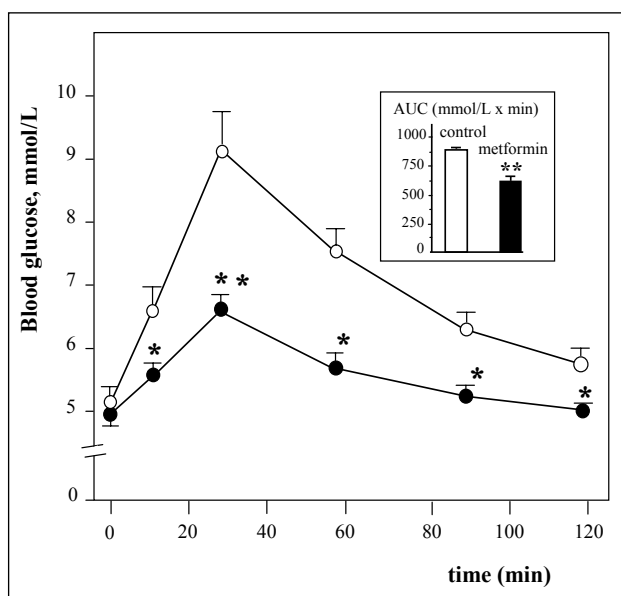

Fig. 5. Effect of metformin on glucose tolerance in rat. Oral glucose tolerance test (OGTT) to 2 g/kg body weight was achieved in 16 h-fasted male Wistar rats. Metformin (300 mg/kg body weight in water, ●) was given by gavage 3 min before glucose. Control (o) received 0.9% NaCl. Evolution of glycemia as a function of time is presented together (insert) with areas under the curves (AUC in mmol/L x min) calculated for each group of rats. ** , significantly different from control value (p<0.01).

jejunal glucose transport induced by metformin is unlikely to have been caused by changes in paracellular permeability. Further cytochalasin B, a specific inhibitor of GLUT2 (28), markedly reduced (-56%; p<0.01) the transport of glucose induced by metformin in line with the involvement of GLUT2 observed under metformin. Furthermore, the effect of metformin

on transmural glucose fluxes were markedly reduced (-77%; p<0.001) by pretreatment with compound C, an inhibitor of AMPK, indicating that activation of AMPK is required for the metformin-induced modulation of glucose flux.

Acute administration of metformin increases glucose tolerance in OGTT

We finally evaluated the impact of acute administration of metformin on glucose tolerance. As depicted in *Fig. 4*, blood glucose concentration after glucose feeding was significantly reduced in rats given metformin (300 mg/kg). This difference was found significant at all time points and the AUC was reduced by 27.6% (p<0.001) after administration of metformin *per os*.

DISCUSSION

The present study shows that metformin induces a fast regulation of the two major intestinal glucose transporters SGLT-1 and GLUT2, through a rapid AMPK phosphorylation. The small intestine was previously reported to be a major site for accumulation and action of biguanides, particularly metformin (2, 29). This transport is ensured by an organic cation transporter from the OCT family that has recently been identified (13). Both metformin and glucose are likely to be transported by enterocyte. However, the exact nature of the effects of metformin on glucose transport and metabolism in these cells remains elusive and some effects of metformin on glucose transporters seem contradictory. In one hand, metformin accumulates in the intestinal mucosa (30) and decreases glucose transport by the intestine in different animal models (30-32). On the other hand, it seems that metformin enhances the abundance of permease GLUT2 at the BBM (33), a mechanism of regulation that is normally set up when the level of glucose uptake increases (34). In line with this increased entry of glucose into enterocyte, metformin can further enhance the utilization of glucose (5, 6) and the anaerobic production of lactate by intestinal cells (2).

An important finding was the discovery that metformin can activate AMPK, a regulatory kinase which is a sensor of energy in most cell (35-37). AMPK is a serine/threonine kinase which acts as a master switch to maintain energy balance by regulating the rates of ATP-consuming and ATP-generating pathways within the cell. It was previously reported that metformin inhibits the glucagon-stimulated hepatic glucose production through activation of AMPK α2 subunit (36). The mechanism of AMPK activation by metformin involves inhibition of ATP synthesis in the complex I of the respiratory chain (38) leading to subtle modifications of AMP binding to AMPK and optimum phosphorylation by upstream kinase LKB1. Although it is clear that metformin activates AMPK, no studies have examined the effect of AMPK activation by metformin on intestinal glucose transporters. Walker *et al*. (39) made the important demonstration in mice jejunum that pharmacological activation of AMPK by 5-amino-4-imidazolecarboxamide ribonucleoside (AICAR) results in a switch in glucose transporters, reducing the ATP-consuming SGLT-1 activity and concomitantly increasing the abundance of GLUT2 at BBM. They also observed that the activation of AMPK occurred 30 min after the exposure of mice jejunum to high concentration of metformin, but they did not examine the effect of metformin on SGLT-1 activity.

In this study, we show that metformin strongly and rapidly decreased glucose-induced abundance of SGLT-1 in BBM, similarly to that previously reported for leptin (16) and resistin-like molecule β (23). As metformin concomitantly increased GLUT2 protein levels in BBM (a hallmark of AMPK activity

(39), we examined the effect of metformin on phosphorylation of AMPKα2. We further demonstrate that mucosal metformin markedly increased the phosphorylation of the catalytic subunit α2 of AMPK in enterocyte. Moreover, metformin effect on glucose transporters can be blocked by compound C, a classical inhibitor of AMPK phosphorylation. Although compound C possibly inhibits other kinases, this makes a possible link between the metformin-induced activation of AMPK and its effect on the expression of glucose transporters. Indeed, the events triggering this pathway may involve first a mobilization of glucose transporters, since non-metabolized sugar 3-O methyl glucose induces the same effects. Finally, when metformin was given orally immediately before an oral load of glucose, it markedly reduced glucose tolerance. Altogether, it can be suggested that metformin modulates the activity of glucose transporters and further regulates glycemia through rapid activation of AMPKα2 subunit in enterocyte, in addition to its concomitant action in muscle (7) and liver (36).

The short-term inhibition of SGLT-1 action was previously found accompanied by a decrease in blood glucose (27). As the inhibition of SGLT-1 by metformin is concomitant with an increase in GLUT2 abundance in BBM, the question arises whether the global effect is to reduce or to stimulate glucose uptake and transport. In our experimental model, transmural glucose transport was significantly increased after metformin, and this increase was mostly mediated by an increase in luminal GLUT2. This increased uptake of glucose is in line with increased metabolism (38) and production of lactate (2) induced by metformin.

It is not known whether redistribution of sugar transporters to or from the apical membrane is sufficient to modify their overall abundance. Nevertheless, the rapid modification of transporter density in the apical membrane has been shown to be associated with significant changes in sugar levels with major consequences for glucose transport and glycemia, and may be consider as an accelerator of absorption (26, 33, 40).

It is noteworthy that D-glucose alone can activate AMPK pathway in enterocyte, as observed in other tissues (15). Fructose, another relevant dietary hexose, is also know to control AMPK activity in small intestine (33, 41). The importance of the AMPK pathway in the process of this autoregulation of intestinal sugar entry has not been studied in enterocyte. Here we show that this pathway is activated in the presence of luminal sugars and modulates sugar absorption independently of glucose metabolism. To this end, sugar transporters SGLT-1 and GLUT2 can be considered as membrane sensors for luminal sugars (22, 42). In addition to the detection of dietary sugars, it can be proposed that this regulation may have a physiological role in limiting the magnitude of the osmotic stress induced by SGLT-1-mediated entry of sugar into enterocyte. Indeed, the intestinal cell must at the same time transport efficiently glucose into the tissues and also deal with a large quantity of water and sodium molecules cotransported with glucose through SGLT-1 (43). The capacity of GLUT2 for cotransport of water molecules is considered to be much smaller -about 10 times- than that of the SGLT-1 (44). In this view, the rapid AMPK-dependent switch of the glucose transport system from sodium-dependent to passive transport could appear as a resolution of these constraints.

Physiological signals for this switch from an energy-dependent glucose entry to a passive transport are not completely understood. It is known that sugars and hormones constitute the major effectors. Substances present in food and beverage like water-soluble green tea extract (45) are also active. Leptin, which acts through control of AMPK in hypothalamus (15, 46), adipose tissue (46) and liver (47), is a potent and rapid inhibitor of the SGLT-1 activity (16) and we recently showed that it modulates the glucose entry through this mechanism (33). AMPK is also a target of angiotensin II and we recently

demonstrated that resistin-like molecule β can also control the shuttling of SGLT-1 from and GLUT2 to the BBM (23). We propose that metformin by a balanced action on glucose transporters abundance in brush border through activation of AMPK, could mimic some mechanisms of action of gut peptides to control peripheral glucose transport. These data give new insight in the mechanism of metformin-induced antihyperglycemia and suggest that rapid control of intestinal glucose transporters at luminal membrane of enterocyte significantly contributes to maintain glucose homeostasis.

REFERENCES

1. Strack T. Metformin: a review. *Drugs Today (Barc)* 2008; 44: 303-314.
2. Bailey CJ, Wilcock C, Scarpello JH. Metformin and the intestine. *Diabetologia* 2008; 51: 1552-1553.
3. Caspary WF, Creutzfeldt W. Inhibition of intestinal amino acid transport by blood sugar lowering biguanides. *Diabetologia* 1973; 9: 6-12.
4. He L, Sabet A, Djedjos S, *et al*. Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. *Cell* 2009; 137: 635-646.
5. Meyer F, Ipaktchi M, Clauser H. Specific inhibition of gluconeogenesis by biguanides. *Nature* 1967; 213: 203-204.
6. Mithieux G, Rajas F, Zitoun C. Glucose utilization is suppressed in the gut of insulin-resistant high fat-fed rats and is restored by metformin. *Biochem Pharmacol* 2006; 72: 1757-1762.
7. Bailey CJ, Puah JA. Effect of metformin on glucose metabolism in mouse soleus muscle. *Diabete Metab* 1986; 12: 212-218.
8. Bailey CJ, Turner RC. Metformin. *N Engl J Med* 1996; 334: 574-579.
9. Cigolini M, Bosello O, Zancanaro C, *et al*. Influence of metformin on metabolic effect of insulin in human adipose tissue in vitro. *Diabete Metab* 1984; 10: 311-315.
10. Stepensky D, Friedman M, Raz I, Hoffman A. Pharmacokinetic-pharmacodynamic analysis of the glucose-lowering effect of metformin in diabetic rats reveals first-pass pharmacodynamic effect. *Drug Metab Dispos* 2002; 30: 861-868.
11. Bell PM, Hadden DR. Metformin. *Endocrinol Metab Clin North Am* 1997; 26: 523-537.
12. Scheen AJ. Clinical pharmacokinetics of metformin. *Clin Pharmacokinet* 1996; 30: 359-371.
13. Zhou M, Xia L, Wang J. Metformin transport by a newly cloned proton-stimulated organic cation transporter (plasma membrane monoamine transporter) expressed in human intestine. *Drug Metab Dispos* 2007; 35: 1956-1962.
14. Wright E, Loo D, Hirayama B, Turk E. Surprising versatility of Na$^+$-glucose cotransporters: SLC5. *Physiology* 2004; 19: 370-376.
15. Minokoshi Y, Alquier T, Furukawa N, *et al*. AMP-kinase regulates food intake by responding to hormonal and

nutrient signals in the hypothalamus. *Nature* 2004; 428: 569-574.

16. Ducroc R, Guilmeau S, Akasbi K, *et al*. Luminal leptin induces rapid inhibition of active intestinal absorption of glucose mediated by sodium-glucose cotransporter 1. *Diabetes* 2005; 54: 348-354.

17. Inigo C, Patel N, Kellett GL, *et al*. Luminal leptin inhibits intestinal sugar absorption in vivo. *Acta Physiol* (*Oxf*) 2007; 190: 303-310.

18. Wong TP, Debnam ES, Leung PS. Involvement of an enterocyte renin-angiotensin system in the local control of SGLT1-dependent glucose uptake across the rat small intestinal brush border membrane. *J Physiol* 2007; 584: 613-623.

19. Cheeseman CI, Tsang R. The effect of GIP and glucagon-like peptides on intestinal basolateral membrane hexose transport. *Am J Physiol* 1996; 271: G477-G482.

20. Ducroc R, Voisin T, El Firar A, Laburthe M. Orexins control intestinal glucose transport by distinct neuronal, endocrine, and direct epithelial pathways. *Diabetes* 2007; 56: 2494-2500.

21. Hirsh AJ, Cheeseman CI. Cholecystokinin decreases intestinal hexose absorption by a parallel reduction in SGLT1 abundance in the brush border membrane. *J Biol Chem* 1998; 273: 14545-14549.

22. Leturque A, Brot-Laroche E, Le Gall M. GLUT2 mutations, translocation, and receptor function in diet sugar managing. *Am J Physiol Endocrinol Metab* 2009; 296: E985-E992.

23. Krimi R, Letteron P, Chedid P, *et al*. Resistin-like molecule-beta inhibits SGLT-1 activity and enhances GLUT2-dependent jejunal glucose transport. *Diabetes* 2009; 58: 2032-2038.

24. Tobin V, Le Gall M, Fioramonti X, *et al*. Insulin internalizes GLUT2 in the enterocytes of healthy but not insulin-resistant mice. *Diabetes* 2008; 57: 555-562.

25. Dyer J, Wood IS, Palejwala A, *et al*. Expression of monosaccharide transporters in intestine of diabetic humans. *Am J Physiol Gastrointest Liver Physiol* 2002; 282: G241-248.

26. Wong TP, Debnam ES, Leung PS. Diabetes mellitus and expression of the enterocyte renin-angiotensin system: implications for control of glucose transport across the brush border membrane. *Am J Physiol Cell Physiol* 2009; 297: C601-C610.

27. Meddah B, Ducroc R, El Abbes Faouzi M, *et al*. Nigella sativa inhibits intestinal glucose absorption and improves glucose tolerance in rats. *J Ethnopharmacol* 2009; 121: 419-424.

28. Axelrod JD, Pilch PF. Unique cytochalasin B binding characteristics of the hepatic glucose carrier. *Biochemistry* 1983; 22: 2222-2227.

29. Jackson RA, Hawa MI, Jaspan JB, *et al*. Mechanism of metformin action in non-insulin-dependent diabetes. *Diabetes* 1987; 36: 632-640.

30. Wilcock C, Bailey CJ. Accumulation of metformin by tissues of the normal and diabetic mouse. *Xenobiotica* 1994; 24: 49-57.

31. Ikeda T, Iwata K, Murakami H. Inhibitory effect of metformin on intestinal glucose absorption in the perfused rat intestine. *Biochem Pharmacol* 2000; 59: 887-890.

32. Lorch E. Inhibition of intestinal absorption and improvement of oral glucose tolerance by biguanides in the normal and in the streptozotocin-diabetic rat. *Diabetologia* 1971; 7: 195-203.

33. Sakar Y, Nazaret C, Letteron P, *et al*. Positive regulatory control loop between gut leptin and intestinal GLUT2/GLUT5 transporters links to hepatic metabolic functions in rodents. *PLoS One* 2009; 4: e7935.

34. Kellett GL, Brot-Laroche E, Mace OJ, Leturque A. Sugar absorption in the intestine: the role of GLUT2. *Annu Rev Nutr* 2008; 28: 35-54.

35. Hardie DG. AMPK: a key regulator of energy balance in the single cell and the whole organism. *Int J Obes* (*Lond*) 2008; 32(Suppl 4): S7-S12.

36. Zhou G, Myers R, Li Y, *et al*. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 2001; 108: 1167-1174.

37. Zou MH, Kirkpatrick SS, Davis BJ, *et al*. Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin in vivo. Role of mitochondrial reactive nitrogen species. *J Biol Chem* 2004; 279: 43940-43951.

38. Penicaud L, Hitier Y, Ferre P, Girard J. Hypoglycaemic effect of metformin in genetically obese (fa/fa) rats results from an increased utilization of blood glucose by intestine. *Biochem J* 1989; 262: 881-885.

39. Walker J, Jijon HB, Diaz H, *et al*. 5-aminoimidazole-4-carboxamide riboside (AICAR) enhances GLUT2-dependent jejunal glucose transport: a possible role for AMPK. *Biochem J* 2005; 385: 485-491.

40. Kellett GL, Brot-Laroche E. Apical GLUT2: a major pathway of intestinal sugar absorption. *Diabetes* 2005; 54: 3056-3062.

41. Kirchner S, Muduli A, Casirola D, *et al*. Luminal fructose inhibits rat intestinal sodium-phosphate cotransporter gene expression and phosphate uptake. *Am J Clin Nutr* 2008; 87: 1028-1038.

42. Moriya R, Shirakura T, Ito J, *et al*. Sodium glucose co-transporter 1 (SGLT1) mediates glucose-induced incretin secretion in mice in vivo. *Am J Physiol Endocrinol Metab* 2009; 297: E1358-E1365.

43. Zeuthen T, Belhage B, Zeuthen E. Water transport by Na+-coupled cotransporters of glucose (SGLT1) and of iodide (NIS). The dependence of substrate size studied at high resolution. *J Physiol* 2006; 570: 485-499.

44. Zeuthen T, Zeuthen E, Macaulay N. Water transport by GLUT2 expressed in Xenopus laevis oocytes. *J Physiol* 2007; 579: 345-361.

45. Park JH, Jin JY, Baek WK, *et al*. Ambivalent role of gallated catechins in glucose tolerance in humans: a novel insight into non-absorbable gallated catechin-derived inhibitors of glucose absorption. *J Physiol Pharmacol* 2009; 60: 101-109.

46. Krskova-Tybitanclova K, Macejova D, *et al*. Short term 13-cis-retinoic acid treatment at therapeutic doses elevates expression of leptin, GLUT4, PPARgamma and aP2 in rat adipose tissue. *J Physiol Pharmacol* 2008; 59: 731-743.

47. Andreelli F, Foretz M, Knauf C, *et al*. Liver adenosine monophosphate-activated kinase-alpha2 catalytic subunit is a key target for the control of hepatic glucose production by adiponectin and leptin but not insulin. *Endocrinology* 2006; 147: 2432-2441.

Author's address: Dr. R. Ducroc, Inserm, U773, Centre de Recherche Biomédicale Bichat-Beaujon, Université Paris Diderot Paris 7, Faculté de Médecine site Bichat, 16 rue Henri Huchard, 75890 Paris cedex 18, France ; Phone: 33-1 5727-7459; Fax: 33-1 5727-7461; E-mail: robert.ducroc@inserm.fr