The effect of leptin on glucose transport was studied in rat jejunal mucosa in Ussing chambers. Leptin was added in the luminal or the serosal compartment before the tissues were challenged with 1, 10, or 50 mmol/l glucose. In response to 10 mmol/l glucose, the increase in short-circuit current (ΔIsc) reached 26.8 ± 2.1 μA/cm². Luminal addition of leptin dramatically decreased glucose-induced Isc (90.5% for 10 nmol/l leptin). Inhibition was maximal after 5 min and dose dependent (IC₅₀ = 0.13 nM). Western blot analysis showed that rapid inhibition of glucose-induced Isc by leptin was associated with a parallel decrease in the abundance of sodium-glucose transporter-1 in brush border membranes. Inhibition by luminal leptin of ΔIsc was prevented by inhibitor of conventional protein kinase C isoforms. Serosal addition of leptin did not decrease glucose-induced Isc within 5 min and reached maximum after 10 min. The effect of leptin from serosal side was blocked by cholecystokinin (CCK) receptor-2 receptor antagonist YM022. Altogether, these data demonstrate that luminal leptin induces rapid inhibition of glucose entry into enterocyte. The slower action of leptin on the serosal side of mucosa seems indirect and is likely mediated by endogenous CCK. They demonstrate that gut leptin is a major regulator of rapid intestinal glucose transport. *Diabetes* 54:348–354, 2005

The hormone leptin, encoded by the *ob* gene, was originally discovered as a weight-reducing protein secreted by adipose cells (1). Indeed, leptin is secreted primarily from adipose tissue, and its circulating levels are correlated with the size of fat stores (2). Leptin released into the bloodstream enters the central nervous system to activate specific receptors in the hypothalamus (3,4). The interaction of leptin with these leptin receptors results in an up- or downregulation of a set of hypothalamic neuropeptides that regulate energy homeostasis (5–7).

Initially characterized as a key adipostatic signal controlling body weight and adiposity, leptin is now considered a multifunctional hormone that also regulates neuroendocrine function, fertility, immune function, and angiogenesis. This current status of leptin is consistent with leptin’s production by various tissues and organs, such as placenta (8), salivary glands (9), and stomach (10,11). The stomach-derived leptin is mainly secreted into gastric juice (10,11), where it remains active even at pH 2 (9). It enters the intestine, where it is detected as free leptin and leptin bound to high molecular weight macro-molecules (12). The demonstration that leptin receptors are present all along the intestine within the brush border (13,14) indicates that leptin entering into the intestinal lumen can initiate biological processes that control functions of the intestinal tract. Indeed, luminal leptin increases the activity of the brush border proton-dependent transporter, PepT1, which enhances the intestinal absorption of oligopeptides (13). Moreover, luminal leptin was reported to increase butyrate uptake through an increase of the amount of the monocarboxylate transporter-1 proteins in Caco-2 cell line (15). However, evidence has been provided that circulating leptin secreted by adipocytes can decrease intestinal triglyceride transport by inhibiting apolipoprotein AIV (16) and sugar transport by inhibiting sodium-glucose transporter-1 (SGLT-1) activity (17). Thus, leptin may be a key molecule in the managing of intestinal absorption of nutrients. However, whether gastric leptin entering the intestinal lumen can modulate glucose absorption is unknown.

Although the small intestine is now considered an insulin-sensitive and gluconeogenic organ (18), it is surprising that most studies focused on the effects of leptin on tissue glucose utilization rather than its effects on intestinal handling of monosaccharides. Indeed, one important function of the small intestine is to absorb glucose. In the preprandial state, glucose transport is an active process that involves cotransport of sugar with sodium ions through SGLT-1. This transporter SGLT-1 is a highly regulated protein that is present in the mucosa from jejunum to colon and is rapidly mobilized in brush border membrane after activation signals (19). The activity of SGLT-1 is enhanced by glucagon-like peptide (GLP) (20,21) and glucose-dependent insulino-tropic polypeptide (22) and reduced by cholecystokinin (CCK) (23) and leptin (17). SGLT-1 also functions as a scavenger and a signal mole-
cule (24). The aim of this study was to determine the effects of luminal (secreted by the stomach) versus circulating (secreted by adipocyte) leptin on the intestinal absorption of glucose and to analyze the cellular mechanisms involved in these effects.

Here, we show that luminal versus serosal leptin rapidly reduces the active intestinal transport of glucose mediated by SGLT1 in a leptin receptor–dependent manner. This inhibitory effect of leptin involves a decreased amount of SGLT1 proteins on the brush border and requires activation of protein kinase C (PKC). We further demonstrate that the delayed inhibitory effect of serosal (versus luminal) leptin on glucose absorption requires activation of CCK-2 receptors.

RESEARCH DESIGN AND METHODS

Animals. Male Wistar rats weighing 220–240 g and male 6- to 8-week-old obese (fa/fa) and lean (Fa/fa) Zucker rats (Charles River Laboratories, L’Arbresle, France) were caged under standard laboratory conditions, with tap water and regular food provided ad libitum, and were on a 12 h light/dark cycle at a temperature of 21–23°C. The animals were treated in accordance with European Community guidelines concerning the care and use of laboratory animals.

Tissue preparation and short-circuit measurement. Rats were fasted for 16 h with water ad libitum. Animals were killed by pentobarbital overdose, and tissue preparation and short-circuit measurement.

RESULTS

We used the polarized system of the Ussing chamber, which permits access to both sides of jejunal mucosa, to characterize the effect of mucosal and serosal leptin on intestinal active glucose transport. After the jejunal mucosa was mounted in the Ussing chamber, the tissue was allowed to reach a steady state (usually 40 min). Jejunal mucosa was then challenged mucosally with 10 mmol/l glucose, and activity of SGLT-1 was followed as Na+-dependent rise in Isc.

Addition of 1, 10, or 50 mmol/l glucose in the mucosal bath of the Ussing chamber induced a rapid (<2 min) and significant rise in Isc. This rise is the result of the Na+-mucosal-to-serosal movement that sustain glucose entry. When tissues were challenged mucosally with 10 mmol/l of the nontransportable sugar, 2-deoxy-D-glucose used as a control, no change in Isc was observed (data not shown).

The addition of 10 mmol/l CCK-8 to the serosal bath resulted in a significant 62.2 ± 9.9% (P < 0.01) decrease (10 min), consistent with earlier data (23). Moreover, incubation of the tissue with GLP-2 at the serosal side resulted in a rapid and strong 50.5 ± 5.9% (P < 0.001) increase in Isc (data not shown), in agreement with previous studies in rat jejenum (21). Thus, our experimental model was suitable for analyzing the effects of leptin on intestinal glucose absorption.

Mucosal leptin induces rapid inhibition of Na+-dependent glucose transport. Addition of leptin to the mucosal side induced a rapid and marked inhibition of glucose-induced Isc. This inhibition reached 94.3 ± 7.2% after 2 min for 10 nmol/l leptin (Fig. 1A). We chose the time point 2 min between leptin addition and glucose challenge for determination of the dose-response effect. As shown in Fig. 1B, addition of leptin to the mucosal side inhibited glucose transport by jejunal mucosa in a concentration-dependent manner. The inhibition was significant with 0.1 nmol/l, maximal inhibition was achieved with 10 nmol/l leptin, and no further inhibition was observed with 100 nmol/l leptin. The concentration that produced a half-maximal inhibition (IC50) of glucose transport was 0.13 nmol/l. The inhibition was also found for 1 and 50 mmol/l glucose (Fig. 1C).

Leptin inhibits transport of the nonmetabolized sugar methyl α-D-glucopyranoside. To examine whether the inhibitory effect of leptin was dependent on the substrate of SGLT-1, we studied the response of jejenum to the nonmetabolizable sugar, methyl α-D-glucopyranoside (MDG). Addition of 10 nmol/l MDG to the mucosal bath induced a rapid and significant rise in Isc (Fig. 1D) that was not significantly different from that induced by glucose (ΔIsc; 25.5 ± 2.1 vs. 25.5 ± 2.1 μA/cm²; n = 5), consistent with previous studies (26). Previous addition of luminal leptin (10 nmol/l) significantly reduced MDG-induced increase in Isc by 76.0 ± 14.4%. This suggests that the luminal leptin inhibition of SGLT-1 function is inde-
dependent of the nature of the substrate and its further metabolism.

**Leptin inhibition of active glucose transport requires functional leptin receptors.** To assess whether leptin has a direct inhibitory effect on glucose transport, we examined the response of jejunal preparation from *fa/fa* rats, which have a strong decrease of function of the leptin receptor (27). Jejunal preparations from the lean counterparts (*Fa/fa*) were also studied as control (Fig. 2). The lean *Fa/fa* rats exhibited a lower Isc response to glucose as compared with Wistar rats. However, both *fa/fa* and lean *Fa/fa* rats exhibited similar Isc responses to 10 mmol/l glucose added to the mucosal side (ΔIsc = 12.1 ± 3.0 and 13.0 ± 4.0 μA/cm²; n = 6–8, respectively). Addition of leptin (10 nmol/l) at the mucosal side failed to inhibit glucose-induced increase in Isc in jejunum from leptin receptor–deficient rats (ΔIsc = 16.4 ± 4.8 μA/cm²; n = 7; P > 0.05 vs. glucose only) but induced a 30% decrease in glucose-induced Isc in jejenum from lean *Fa/fa* rats (ΔIsc = 8.0 ± 1.5 vs. 13.0 ± 4.0 μA/cm²; n = 3).

**Luminal leptin reduces SGLT-1 abundance in the BBM.** To assess the mechanism of mucosal leptin inhibition, we analyzed the abundance of SGLT-1 protein in the BBM after glucose challenge in the presence or absence of leptin. As shown in Fig. 3, glucose (10 mmol/l) increased the amounts of SGLT-1 protein in the BBM by 3.7-fold. Previous 3-min treatment with 10 nmol/l leptin in the jejunal lumen prevented the glucose-induced increase in the abundance of BBM SGLT-1 (Fig. 3B and C). No change in the amount of SGLT-1 protein was found in total extract protein after jejunal loops challenge with 10 mmol/l glucose challenge, in either the presence or the absence of leptin in vivo. This suggests that luminal leptin inhibits glucose transport by preventing translocation of cytosolic preformed SGLT-1 to cell membranes.

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**FIG. 1.** A: Effect of luminal leptin on glucose-induced Isc. A typical recording of Isc increase in response to luminal challenge by 10 mmol/l glucose. Rat jejunal mucosa was mounted in Ussing chamber, and the increase in Isc was studied at steady state. Electrogenic Na⁺ transport was followed as an index of the active glucose transport by cotransporter SGLT-1. Note the occurrence of the inhibition 2 min after addition of leptin (10 nmol/l) in the mucosal bath. Electrogenic chloride secretion in response to carbachol (100 μmol/l) was used as a control. B: Dose-response for luminal leptin inhibition of glucose-induced Isc in rat jejunum. Leptin was added in the mucosal bath 3 min before tissues were challenged with 10 mmol/l glucose. Values for Isc were standardized to control values and expressed in percentage of controls. Each point represents the mean ± SE of four to eight noncumulative values from five separate experiments. C: Inhibition of glucose-induced Isc by luminal leptin: effect of different concentrations of glucose. Leptin was added in the mucosal bath 3 min before tissues were challenged with 1, 10, or 50 mmol/l glucose. Each point represents the mean ± SE of five to seven noncumulative values from five separate experiments. D: Effect of luminal leptin on Isc induced by the nonmetabolized sugar MDG. Leptin was added in the mucosal bath 2 min before tissues were challenged with 10 mmol/l MDG, a substrate for SGLT-1 that is not a metabolized sugar. **Significantly different from control (P < 0.01).
Leptin inhibition of SGLT1 involves classic PKC isoform. PKC is a family of isozymes that are grouped into three subclasses (conventional, atypical, and novel) on the basis of the domain composition of the regulatory moiety (28). Cellular regulation of SGLT-1 has been shown to involve phosphorylation and turnover of PKβII, mediated by phosphatidylinositol 3-kinase (25). We therefore analyzed the effects of PKC inhibitors on leptin inhibition of glucose transport. Previous addition in the mucosal bath of 1 mmol/l G6976, a PKC inhibitor of classic isoforms, completely prevented the inhibition of glucose transport induced by mucosal leptin. However, rottlerin (5 μmol/l), an inhibitor of novel isoforms of PKC, had no inhibitory effect on glucose-induced Isc by leptin (Fig. 4).

Serosal leptin reduces glucose transport. For comparison purposes, we studied the effects of serosal versus mucosal leptin on glucose-induced Isc. As shown in Fig. 5 (insert), when 10 nmol/l leptin was introduced into the serosal bath, surprisingly, no inhibition of glucose transport could be observed over a 2-min period as compared with the marked inhibition that occurred when it was added in the mucosal bath (Fig. 5).

Kinetic studies revealed a time-dependent delay in the inhibition of glucose transport when leptin was added to the serosal versus mucosal compartment (Fig. 5). Indeed, when added to the mucosal bath, the leptin inhibition of glucose transport occurred as rapid as 2 min (94 ± 7% inhibition), was maximal 5 min after leptin, and then slightly decreased to reach 44 ± 19% inhibition 20 min after leptin addition. However, when added to the serosal bath, no leptin inhibition of glucose transport occurred after 2 min. This inhibition was significant after 5 min, maximal after 10 min, and rapidly decreased after 20 min. Thus, luminal leptin is more rapid than circulating leptin to inhibit intestinal active glucose transport.

Serosal leptin inhibition of glucose transport requires activation of CCK-2 receptor. The marked difference in the kinetics of inhibition between serosal and mucosal leptin is suggestive for the implication of an intermediate molecule. Because CCK has been shown to inhibit SGLT-1 activity, we examined the effects of leptin in the presence or absence of CCK-1 or -2 receptor antagonists.

Previous treatment of jejunal preparation with YM022 (1 nmol/l), a CCK-2 receptor antagonist, completely prevented the inhibitory effect of serosal leptin on glucose transport, whereas 1 nmol/l L364718, a CCK-1 receptor antagonist, did not (Fig. 6). However, none of the antagonists modified the rapid inhibition effect of mucosal leptin. Altogether, this suggests that the delayed inhibitory effect of serosal leptin is likely to involve endogenously released CCK acting at the CCK-2 receptor.

**DISCUSSION**

In this study, we demonstrated that leptin acting at a luminal surface of jejunal enterocytes potently inhibits the active component of intestinal glucose transport mediated by SGLT-1. The results herein point to a new role for gastric leptin as a key molecule in the regulation of intestinal absorption of dietary sugars.

Intestinal glucose absorption involves two components (rev. in 24), including active transport mediated by SGLT-1 (29). In the physiological state of short fasting and for
enteral concentration of glucose less than \( \sim 40 \text{ mmol/l} \), intestinal cotransporter SGLT-1 is the first route of entry of glucose. The exit of glucose out of the enterocyte is mediated by GLUT2. In addition, transport of glucose by SGLT-1 triggers a rapid activation and recruitment of passive GLUT2 to the BBM (24). In normal conditions, plasma glucose concentration varies from 4.5 to 6 mmol/l, even during ingestion of meal, indicating that the absorption process of dietary sugars across the small intestine is a highly regulated process that is essential for the maintenance of glucose homeostasis. In this connection, the activity of SGLT-1 was reported to be under the control of various hormonal factors. Thus, glucose-dependent insulinotropic polypeptide produced by the duodeno-jejunal endocrine K-cells (30) and GLP-2 from pro-glucagon in endocrine L-cells (31) is able to upregulate SGLT-1 transport activity in vivo, leading to an increased intestinal absorption of glucose (21,22). However, the intestinal peptide CCK secreted by the duodenal endocrine I-cells has been shown to decrease intestinal hexose absorption through a reduction in the amount of BBM SGLT-1 pro-

**FIG. 4.** Effect of inhibitors of PKC isoforms on leptin inhibition of active glucose transport. Jejunal mucosa isolated in Ussing chamber was challenged with 10 mmol/l glucose, and increase in Isc was measured as a index of active glucose transport in control conditions (CTRL) 3 min after luminal addition of 10 mmol/l leptin or previous treatment with 1 \( \mu \text{mol/l} \) Go 6976 (left) or 5 \( \mu \text{mol/l} \) rottlerin followed by addition of leptin. Go 6976 and rottlerin are inhibitors of conventional and novel isoforms of PKC, respectively. Data are presented as means \( \pm \text{SE} \) of increase in Isc expressed in \( \mu \text{A/cm}^2 \). *\( P < 0.01 \) vs. CTRL; **\( P < 0.01 \) vs. leptin.

**FIG. 5.** Comparative action of mucosal versus serosal leptin. *Insert:* Effect of serosal leptin (S. Lep.) versus mucosal leptin (M. Lep.) on glucose-induced Isc after 2 min. Kinetics of the inhibitory effect of leptin. Leptin (10 mmol/l) was added in either the mucosal or the serosal reservoir of Ussing chamber, and the tissues were challenged by 10 mmol/l glucose after different times from 2 up to 20 min. Glucose transport is measured as the \( \text{Na}^+ \)-dependent increase in Isc (\( \Delta \text{Isc in % of controls} \)). Note the marked difference in the profiles of the response curves. ****\( P < 0.01 \) vs. CTRL.

**FIG. 6.** Basolateral leptin action requires active CCK-2 receptors. Effect of CCK receptor subtype blockers on inhibition of glucose transport induced by serosal leptin. Rat jejunal mucosa mounted in Ussing chambers were pretreated for 10 min with either CCK-1 receptor blocker L-364718 or CCK-2 receptor blocker YM022 (1 mmol/l). Leptin was added in the serosal bath, and the tissue was challenged by 10 mmol/l glucose after another 10 min. Data are presented as net increase in Isc (\( \Delta \text{Isc} \)) expressed in \( \mu \text{A/cm}^2 \). **\( P < 0.01 \) vs. CTRL; ***\( P < 0.01 \) vs. leptin.
teins (23). All of these hormones that are secreted in the bloodstream are likely to exert their effects via activation of their specific receptors at the basolateral side of the enterocytes.

Although it is likely that leptin has an indirect role on regulation of intestinal glucose absorption because it stimulates intestinal release of CCK (12) and GLP-1 (32), both involved in the control of active glucose absorption, only one group studied the direct effects of leptin on this function. Indeed, leptin was reported to inhibit SGLT-1 transport activity leading to reduction of intestinal absorption of galactose by rat jejunal rings (17, 33). In these studies, the experimental model used did not allow the authors to discriminate between serosal and mucosal action of leptin. Thus, it was unknown whether gastric leptin entering the intestinal lumen is able to modulate glucose absorption. In the present study, we clearly demonstrate that mucosal leptin markedly decreases the active component of glucose transport mediated by SGLT-1, arguing for a new intestinal function of gut leptin. The concentration of 10 nmol/l glucose was used as a physiological concentration that induces insulin secretion in pancreatic islet cells (34) and that remains largely under the maximum capacity of transport of SGLT-1 (24). We found that the luminal action of leptin was rapid and concentration dependent with an IC₅₀ value of 0.1 nmol/l, close to that found for leptin inhibition of insulin release from pancreatic cells (34). Our finding that luminal leptin inhibited nonmetabolized sugar MDG entry into the enterocytes further indicates that leptin inhibits SGLT-1 activity independent of the substrate and of its metabolism.

The decrease in SGLT1 activity by luminal leptin is consistent with previous studies describing the expression of leptin receptors on the apical side on the enterocytes in the jejunum (13, 14). They suggest that luminal leptin can bind to apical leptin receptor on jejunal enterocytes to decrease the activity of SGLT-1. The failure of mucosal leptin to affect SGLT-1 transport activity in jejunum from the leptin receptor–deficient rats indicates that functional leptin receptors are required for this effect. The genetically obese fa/ fa rats carry a missense mutation that results in an amino acid substitution at position 269 (Gln-Pro) within the extracellular domain of the leptin receptor (35) and that leads to strong decrease of function of the receptor (36). It is noteworthy that in basal conditions, jejunal preparations of Zucker rats exhibited a lower Isc response to glucose than preparations from Wistar rats. We have no clear explanation for this difference, but it is likely to reflect differences in the genetic background of the two strains.

The mechanisms that are responsible for leptin inhibition of SGLT-1 function may involve modulation of its intrinsic activity and/or abundance of SGLT-1 proteins in the BBM. We demonstrated that leptin inhibition of glucose transport activity is associated with a marked reduction in the amount SGLT-1 proteins in the BBM. This indicates that leptin effects involve a decreased insertion of SGLT-1 molecules recruited from the preformed intracellular pool into the BBM. However, this does not exclude that leptin may also affect the affinity of the transporters, as previously observed (17, 23).

That luminal leptin inhibits intestinal SGLT-1 activity can be put forward with our previous studies showing that luminal leptin increases intestinal transport of d- and tripeptides via the brush border PepT-1 transporter in vivo in rat (13) and enhances the uptake of butyrate mediated by monocarboxylate transporter-1 in vitro in colon cancer cells (15). Taken together, it suggests that in physiological conditions, the rapid release of gastric leptin that occurs during a meal (10) and that enters the intestine (12) is a key factor in the handling of nutrients absorption.

We also observed that an inhibitor of conventional isoforms of PKC but not of novel isoforms reverses the leptin inhibition of glucose transport, arguing for the involvement of the conventional isoform of PKC. This is in line with data showing that activation of PKC is more relevant than PKA activation in the inhibition of galactose absorption by leptin (33). There is a family of PKC isozymes with different sensitivities to activation (conventional, atypic, and novel) (28). Activation of PKC within cells is a complex process that includes phosphorylation and translocation of cytosolic PKC to membranes where there is catalytic activity as well as involvement of PKC-binding proteins that may further target the activated enzyme to specific sites (28). It therefore is likely that PKC may be involved in the leptin reduction of recruitment SGLT-1 molecules from the intracellular preformed pool into the BBM. In pancreatic islets, leptin was also shown to target the PLC-PKC regulatory pathway to constrain insulin secretion (34).

An unexpected but interesting finding is that serosally applied leptin is less rapid than mucosal leptin to inhibit SGLT-1 transport activity. This difference may reflect gastric versus adipocyte regulation of glucose homeostasis. It suggests that gut leptin may control the absorption of sugars during the meal, whereas circulating leptin from adipose tissue controls long-term regulation of tissue glucose utilization. The slow time course effect for serosal leptin may also reflect a long diffusion pathway before reaching its receptors at the mucosal side. We found that serosal leptin inhibition of glucose transport is completely prevented by blockade of CCK-2 receptor at the serosal side. Because the CCK-2 receptor antagonist did not affect the luminal effect of leptin, we conclude that inhibition of SGLT-1 activity by serosal leptin is likely mediated by CCK and requires activation of CCK-2 receptors. Such an effect is consistent with our previous demonstration that endocrine I-cells are responsive to peripheral leptin (12) and with the reported inhibitory effect of CCK on SGLT-1 activity in rat small intestine (23). It could be of importance in the pathological state in which mesenteric adipocytes are stimulated to secrete leptin (37).

Leptin can trigger secretion of CCK-8 by duodenal jejunal endocrine I-cells (12) and can also stimulate release of GLP-1 from intestinal endocrine L-cells (32). After their release in the blood circulation, CCK and GLP-1 can exert their inhibitory and stimulatory effects, respectively, on activity of SGLT-1, contributing to a balanced glucose transport by enterocyte. Here, we demonstrate that in addition to this integrated regulating role, leptin can inhibit SGLT-1, indicating that leptin is playing a conductor role in peripheral glucose homeostasis.

Transepithelial intestinal glucose transport is generally assumed to play a key function in providing energy sub-
strate to multiple tissues. Because the small intestine is now considered as a new insulin-sensitive organ in which major regulatory genes of gluconeogenesis are expressed (18) and because the expression of intestinal SGLT-1 is dramatically increased in diabetic humans (38), our current findings argue for a physiological implication of gut leptin both in the maintenance of glucose and in metabolic disorders.

In summary, this study demonstrates that luminal leptin decreases, in a leptin receptor–dependent manner, the active component of intestinal absorption of glucose mediated by the brush border SGLT-1. This effect involves the activation of classic PKC isoforms and inhibition of the recruitment of the intracellular SGLT-1 proteins into the BBM. It also shows that luminal leptin is more rapid than serosal leptin, which requires activation of CCK-2 receptor for inhibition of SGLT-1 transport activity.

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