Tripeptides of RS1 (RSC1A1) Inhibit a Monosaccharide-dependent Exocytotic Pathway of Na\(^{+}\)–D-Glucose Cotransporter SGLT1 with High Affinity*

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The human gene RSC1A1 codes for a 67-kDa protein named RS1 that mediates transcriptional and post-transcriptional regulation of Na\(^{+}\)-D-glucose cotransporter SGLT1. The post-transcriptional regulation occurs at the trans-Golgi network (TGN). We identified two tripeptides in human RS1 (Gln-Cys-Pro (QCP) and Gln-Ser-Pro (QSP)) that induce posttranscriptional down-regulation of SGLT1 at the TGN leading to 40–50% reduction of SGLT1 in plasma membrane. For effective intracellular concentrations IC\(_{50}\) values of 2.0 nM (QCP) and 0.16 nM (QSP) were estimated. Down-regulation of SGLT1 by tripeptides was attenuated by intracellular monosaccharides including non-metabolized methyl-\(\alpha\)-D-glucopyranoside and 2-deoxyglucose. In small intestine post-transcriptional regulation of SGLT1 may contribute to glucose-dependent regulation of liver metabolism and intestinal mobility. QCP and QSP are transported by the H\(^{+}\)-peptide cotransporter PepT1 that is colocalized with SGLT1 in small intestinal enterocytes. Using coexpression of SGLT1 and PepT1 in Xenopus oocytes or polarized Caco-2 cells that contain both transporters we demonstrated that the tripeptides were effective when applied to the extracellular compartment. After a 1-h perfusion of intact rat small intestine with QSP, glucose absorption was reduced by 30%. The data indicate that orally applied tripeptides can be used to down-regulate small intestinal glucose absorption, e.g. in diabetes mellitus.

Glucose absorption in the small intestine is mediated by the combined action of two glucose transporters in the enterocytes: the sodium-dependent D-glucose cotransporter SGLT1 in the brush-border membrane and the sodium-independent glucose transporter GLUT2 in the basolateral membrane (1, 2). It plays a pivotal role for maintenance of blood glucose concentration (3) and glucose and fat metabolism in the liver (4, 5). In addition, small intestinal glucose uptake triggers glucose-dependent mechanisms that are mediated by glucose sensing neurons in the intestinal wall, for example, the regulation of intestinal motility (6–9).

The absorption of glucose in small intestine changes dramatically during development (10). It is regulated in response to diet and during food intake via changes of expression, location, and activity of SGLT1 and/or GLUT2 (2, 10, 11, 12). Expression of SGLT1 is influenced by \(\beta\)-adrenergic innervation (13), glucagon-like peptide 2 (14), and cholecystokinin (15). Cyclic AMP, protein kinase A, protein kinase C (PKC),\(^{3}\) and phosphoinositol 3-kinase are involved in the regulatory pathways (13, 14, 16). It has been shown that SGLT1 can be regulated by changes in transcription (12, 17, 18), mRNA stability (19), intracellular trafficking (14, 16, 20, 21), and transporter activity (22). However, the individual regulatory pathways, their cross-talk, and their physiological importance are not understood.

Previously, we identified the intracellular protein RS1 (human gene RSC1A1) that participates in the transcriptional and post-transcriptional regulation of SGLT1 (21, 23–28). RSC1A1 is an intronless single copy gene that was only detected in mammals and codes for 67–68-kDa proteins in human, pig, rabbit, and mouse that exhibit about 70% amino acid identity. RS1 has a broad tissue distribution, including renal proximal tubules, small intestinal epithelial cells, hepatocytes, and neurons (23, 28, 29). In LLC-PK\(_{1}\) cells RS1 was located to the intracellular side of the plasma membrane, at the trans-Golgi network and within the nucleus (30). Importantly, the amount and distribution of RS1 were dependent on the state of confluence (30). Whereas subconfluent LLC-PK\(_{1}\) cells contained large amounts of RS1 protein and exhibited pronounced nuclear location of RS1, the amount of RS1 was decreased in confluent LLC-PK\(_{1}\) cells and RS1 did not distribute into nuclei (30). The observed changes in distribution correlated with functional data showing that RS1 down-regulates the transcript-

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\(^{3}\) The abbreviations used are: PKC, protein kinase C; TGN, trans-Golgi network; AMG, methyl-\(\alpha\)-D-glucopyranoside; BTXB, botulinum toxin B; DPBS, Dulbecco’s phosphate-buffered saline; BFA, brefeldin A; QCPac, acetylated QCP; QSPac, acetylated QSP; aa, amino acids; HPLC, high pressure liquid chromatography; hRS1, human RS1; PM, phorbol 12-myristate 13-acetate; MES, 4-morpholineethanesulfonic acid.
Inhibition of SGLT1 Expression by Tripeptides

In Xenopus oocytes, expression of SGLT1 was inhibited when human RS1 was coexpressed (23, 24, 27) or when purified RS1 protein was injected into SGLT1 expressing oocytes shortly before uptake measurements (21). This indicated that hSGLT1 was inhibited by hRS1 on the posttranscriptional level. We showed that this posttranscriptional inhibition occurred within 30 min and was due to blockage of the release of SGLT1 containing vesicles from the TGN (21, 30). This posttranscriptional down-regulation of SGLT1 by RS1 was increased by PKC and modulated by intracellular methyl-α-d-glucopyranoside (AMG) (21). Interestingly RS1 was found to be associated with a 28-kDa protein called the IRIP protein that is up-regulated in kidneys after ischemia and reperfusion (31). The physiological and potential biomedical importance of RS1 was demonstrated by targeted disruption of the Rsc1A1 gene in mice (26). Mice without RS1 showed an increased amount of SGLT1 protein in small intestinal brush-border membranes that was correlated with an increased capacity for small intestinal glucose absorption. Furthermore, mice without RS1 developed an obese phenotype.

In the present study we tried to identify the domain of human RS1 (hRS1) that is responsible for inhibition of the release of SGLT1 containing vesicles from TGN. Two tripeptides of hRS1 were identified that are high affinity inhibitors of hSGLT1 at the TGN. Down-regulation of hSGLT1 by the tripeptides was modulated by intracellular monosaccharides including AMG that is not metabolized, and 2-deoxyglucose, which does not interact with hSGLT1. We showed that the tripeptides are substrates of the small intestinal peptide transporters PepT1 (32) and that they were capable to down-regulate SGLT1 in rat small intestine after application to the intestinal lumen.

EXPERIMENTAL PROCEDURES

Animals

Male rats (Wistar, Crl:WI) purchased from Charles River (Sulzfeld, Germany) were housed in groups of 3–4 animals in makrolon cages with free access to Standard Diet 3433 (Provimi Kliba, Kaiseraugst, Switzerland) and drinking water. The animals were kept under 12-h light/dark cycle at room temperature of 22 ± 2 °C and a relative humidity of 45–75%. Body weight at the day of surgery was 473 ± 17 g (n = 7). *Xenopus laevis* frogs were obtained from Nasco (Ft. Atkinson, WI). Animals were handled in compliance with institutional guidelines and German laws.

Materials

Methyl-α-d-[14C]glucopyranoside ([14C]AMG, 11.7 GBq/mmol) was obtained from Amersham Biosciences. Botulinum toxin B (BTXB) and Dulbecco’s phosphate-buffered saline (DPBS) were supplied by Sigma. Brefeldin A (BFA) was obtained from Calbiochem (Schwalbach, Germany), protein G conjugated with horseradish peroxidase from Bio-Rad, and prestained molecular weight markers from MBI Fermentas (St. Leon-Rot, Germany). Other chemicals and enzymes were purchased as described (23, 25, 33).

Antibody Against Human SGLT1 (hSGLT1)

Polyclonal antibody against a peptide of hSGLT1 (34) (amino acids 581–599, QEGPKETIEITQVPEKKK-C) was raised in rabbits. The peptide with the C-terminal cysteine was coupled to ovalbumin with m-maleimidobenzyol-N-hydroxysuccimide ester. Affinity purification of the antisera was performed on the antigenic peptide coupled to polyclarlamide beads by using the Sulfolink kit from Pierce. The specificity of the antibody was verified by Western blots on cells that were transfected with hSGLT1 and by immunohistochemistry on human small intestine.

Cloning of Truncated Variants of hRS1

Variants of hRS1 were made on the basis of wild type hRS1 cloned into Apal and Xhol sites of the oocyte expression vector pRSSP (21).

Variant I–251—PCR was performed using pRSSP plasmid-specific forward primer and a reverse primer at the corresponding position of hRS1 containing stop codon and the XhoI restriction site. The amplicate was digested with Apal and XhoI and inserted into pRSSP vector.

Variants 326–617, 407–617, and 534–617—PCR was performed using pRSSP plasmid-specific reverse primer and forward primers at corresponding positions of hRS1. Forward primers were provided with initiation codon and Apal site. After Apal/XhoI digestion isolated fragments were inserted into pRSSP vector.

Variants 350–425 and 396–425—PCR was performed using primers to the corresponding positions of hRS1. Forward primers contained initiation codon and XhoI site, whereas reverse primers contained stop codon and the XhoI site. PCR amplified fragments were cloned into Apal/XhoI-digested pRSSP vector.

Variant 251–617—Plasmid pRSSP/hRS1 was cut with Apal, the adaptor Apal/ClaI containing the initiation codon was added, and digestion with ClaI was performed to remove excess ligated adaptor and to cut hRS1 at the ClaI site of the coding region. The 5’ terminal fragment of hRS1 was removed and the plasmid was self-ligated resulting in construct 251–617.

Variant 251–488—Variant 251–617 was cut with EcoRI and XhoI, the 5’ overhangs were filled in using Klenow fragment of DNA polymerase I, and the adaptor Spel provided with the stop codon was added. After ligation the mixture was digested with Spel to remove excess ligated adaptor, the 3’ terminal fragment of hRS1 was removed, and the plasmid was self-ligated resulting in construct 251–488.

Variant 407–415—Two complementary phosphorylated oligonucleotides were designed to cover the desired region of hRS1: 5’-pCATGCAGAATGAACTGTCACAAAGCTCTC-ATGAC-3’ and 5’-pTCGAGTCAGAAGCTGGAGACATGTG-TCATTCTCAGTGCCC-3’. The oligonucleotides contained the initiation and stop codon (bold letters) and were provided with Apal and XhoI cohesive ends (underlined). The oligonucleotides were annealed and ligated into Apal/XhoI-digested pRSSP vector. All PCR-derived parts of the truncated variants were sequenced to rule out PCR errors.
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Synthesis of Peptides

For solid phase synthesis of N-acetylated and nonacetylated peptides we employed the Fmoc (N-(9-fluorenylmethoxycarbonyl) strategy in a fully automated synthesizer (ABI 433, Applied Biosystems, Weiterstadt Germany) (35, 36). Peptide chain assembly was performed using in situ activation of amino acid building blocks by 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. Peptides were purified by preparative HPLC on Kromasil 100-10-C18 reverse phase columns using linear gradients of 0.1% trifluoroacetic acid in water and 80% acetonitrile in water as eluents. The purified material was lyophilized and characterized with analytical HPLC and laser desorption mass spectrometry (Reflex II, Bruker, Bremen Germany).

In Vitro Synthesis of cRNA

For injection into *X. laevis* oocytes, m7G(5')G-capped cRNA was prepared, purified, and stored as described earlier (23). To prepare sense cRNA from hRS1 (24), fragments of hRS1, hSGLT1 (34), or hPepT1 (37), the respective purified plasmids were linearized with EcoRI (hSGLT1), NotI (hPepT1), or MluI (hRS1 and hRS1 fragments). cRNA was synthesized using T3 polymerase (hSGLT1), SP6 polymerase (hRS1, fragments of hRS1), or T7 polymerase (hPepT1) as described earlier (27). cRNAs were prepared employing the "mMESSAGE mMACHINE" kit (Ambion) using sodium acetate precipitation. cRNA concentrations were estimated from ethidium bromide-stained agarose gels using polynucleotide marker as standards.

Expression of Transporters and hRS1 in Oocytes of *X. laevis*

Mature female *X. laevis* were anesthetized by immersion in fresh water containing 0.1% 3-aminobenzoic acid ethyl ester. Oocytes at the stages V and VI were obtained by partial ovariectomy and treated overnight with 1 mg ml−1 collagenase I in Ori buffer (5 mM HEPES-Tris, pH 7.6, 110 mM NaCl, 3 mM KCl, 1 mM MgCl₂, and 2 mM CaCl₂). The oocytes were washed twice with Ca²⁺-, free Ori buffer and kept at 16 °C in modified Barth's solution (15 mM HEPES, pH 7.6, 88 mM NaCl, 1 mM KCl, 0.3 mM Ca(NO₃)₂, 0.4 mM CaCl₂, 0.8 mM MgSO₄) containing 12.5 μg ml⁻¹ gentamycin. Selected oocytes were injected with 25 nl of water containing cRNAs (2.5 ng of hSGLT1, 10 ng of hPepT1, 7.5 ng of hRS1 or fragments of hRS1). For expression, injected oocytes were kept for 3 days at 16 °C in modified Barth's solution with gentamycin. Non-injected oocytes served as controls. In some experiments PKC was stimulated by incubation of the oocytes for 2 min with 1 μM phorbol 12-myristate 13-acetate (PMA).

Injection of Peptides, Monosaccharides, and Biochemicals into Oocytes

Three days after cRNA injection into oocytes before flux experiments were started, 25 nl of K-Ori buffer (5 mM HEPES, pH 7.6, 100 mM KCl, 3 mM NaCl, 2 mM CaCl₂, 1 mM MgCl₂) containing peptides, 0.5 pmol of sn-1,2-dioctanoylglycerol (stimulation of PKC), 2 ng of BTXB (inhibition of exocytosis), and/or 0.5 pmol of BFA (inhibition of vesicle budding from the TGN) were injected. Oocytes were incubated for 1 h at room temperature in Ori buffer and tracer uptake measurements were performed. To investigate the influence of intracellular monosaccharide on tripeptide-dependent down-regulation of hSGLT1, different concentrations of sugars or polyolcohols with or without 75 pmol tripeptides were injected into hSGLT1 expressing oocytes 1 h before uptake measurements were started. Intracellular concentrations of injected compounds were estimated by assuming an internal distribution volume of 0.4 μl (38).

Uptake Measurements in Oocytes

For AMG uptake measurements, hSGLT1 expressing oocytes or non-cRNA-injected control oocytes were incubated for 15 min at room temperature in Ori buffer containing 1.75 or 50 μM [¹⁴C]AMG. Oocytes were washed four times in ice-cold Ori buffer containing 1 mM phlorizin, and single oocytes were solubilized in 5% (w/v) SDS and analyzed for radioactivity by scintillation counting. Uptake of [¹⁴C]AMG was corrected for uptake measured in non-cRNA-injected control oocytes.

Current and Capacitance Measurements

Parallel measurements of membrane current (Iₘ) and membrane capacitance (Cₘ) were performed using an EPC-05 amplifier controlled by PULSE and X-Chart software provided by HEKA Electronics (Lambrecht, Germany). Iₘ measurements were performed using the two-electrode voltage clamp method and Cₘ was measured using a previously described paired ramps approach (39). For Iₘ and Cₘ measurements in oocytes expressing hSGLT1, oocytes were superfused at room temperature with Ori buffer and clamped to −50 mV. For Iₘ measurements in oocytes expressing hPepT1, oocytes were superfused with modified Barth's solution adjusted to pH 6.5, and clamped to −60 mV.

Cultivation of Caco-2 Cells, Incubation with Tripeptides, and Uptake Measurements

The cell line Caco-2 was acquired from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cells were grown on Petri dishes (37 °C, 5% CO₂) in complete Dulbecco's modified Eagle's medium containing 25 mM d-glucose. Nine days after reaching confluence cells were detached by incubation with DPBS containing 0.5 mM EDTA and 20 mM d-glucose, collected by 10-min centrifugation at 1000 × g, and washed with DPBS containing 20 mM d-glucose (DPBS-G). To test effects of tripeptides, cells were incubated for 1 h at 37 °C in DPBS-G that was adjusted to pH 6.5. Incubation at pH 6.5 was performed in the absence and presence of tripeptides. Before transport measurements were started cells were washed two times with DPBS. For uptake measurements cells were incubated for 10 min at 37 °C in DPBS containing 50 μM [¹³C]AMG or 50 μM [¹⁴C]AMG plus 0.5 mM phlorizin. Uptake was terminated by the addition of ice-cold DPBS containing 1 mM phlorizin (stop solution). Cells were washed 3 times with stop solution, solubilized, and analyzed for radioactivity. For each experiment, five measurements were performed per experimental condition.
Measurement of SGLT1 Expression by Tripeptides

A modified version of a previously described perfusion model was used (40). Fasted male rats were anesthetized with pentobarbital (Narcoren, Merial GmbH, Halbergmoos, Germany, 60 mg/kg intraperitoneal). Their body temperature was maintained with a heated pad. Following a laparotomy, a 25-cm long segment of the small intestine starting 1 cm distally of the stomach was isolated and cannulated at both ends. For pre-treatment with tripeptide, the small intestinal segments were perfused for 1 h (recirculating system, 4 ml/min, 38 °C) with DPBS, pH 6.5. The perfusion was performed in the absence and presence of 15 mM QSP. Thereafter, perfusion was switched to single pass perfusion with DPBS (pH 7.4, 38 °C) containing 10 μM D-glucose, and after 20 min to DPBS containing 50 μM D-glucose. Intestinal efflux samples were collected at 2.5-min intervals. D-Glucose determination in the efflux samples was performed using the Amplex® Red Glucose GO assay kit (Invitrogen) adapted for measurements of low D-glucose concentrations.

Isolation of Plasma Membrane from X. laevis Oocytes

Non-injected oocytes and oocytes injected with hSGLT1 cRNA were incubated for 3 days and oocytes without follicular epithelial cells were selected. hSGLT1 expressing oocytes were injected with 25 nl of K-Ori buffer without and with 75 pmol of acetylated QCP (QCPac) and kept for 1 h at room temperature in modified Barth’s solution. Thereafter oocytes were incubated for 15 min at room temperature in Ori buffer containing 50 μM AMG, and washed twice with Ori buffer. Isolation of plasma membranes from oocytes was performed as described (41). For each experimental condition experiments were performed with 10 oocytes. Defolliculated oocytes were rotated for 30 min at 4 °C in MES buffer (20 mM MES, pH 6.0, 80 mM NaCl) containing 1% (w/v) colloidal silica (Ludox Cl from Sigma). Then oocytes were washed two times with MES buffer, rotated 30 min at 4 °C in MES buffer containing 0.1% polyacrylic acid (Sigma), and washed two times with Ori buffer. Oocytes were homogenized in 1.5 ml of homogenization buffer (HbA) (20 mM Tris-HCl, pH 7.4, 100 mM dithiothreitol, 2% (v/v) SDS, and 7% (v/v) glycerol, 0.1% (w/v) bromphenol blue).

SDS-PAGE and Western Blotting

Protein concentration was determined according to Bradford (42). For SDS-PAGE, membrane protein samples were pretreated for 45 min at 37 °C in SDS-PAGE sample buffer. Electrophoresis and Western blotting were performed as described (33). Proteins separated by SDS-PAGE were transferred by electroblotting to polyvinylidene difluoride membrane. For Western blot analysis, the blots were blocked with blocking buffer (DPBS, pH 7.4, 2% bovine serum albumin, 0.1% Tween 20), followed by a 2-h incubation at room temperature with affinity purified antibody against hSGLT1 diluted 1:20,000 in blocking buffer. After washing, the blots were incubated for 2 h at room temperature with horseradish peroxidase-conjugated protein G (Bio-Rad) diluted in blocking buffer. The immunoreaction was visualized by enhanced chemiluminescence using the ECL system (Amersham Biosciences Europe).

RESULTS

hRS1-derived Tripeptides Inhibit AMG Uptake Expressed by hSGLT1—Previously we described that uptake of AMG into oocytes of X. laevis expressing hSGLT1 was decreased when hRS1 was coexpressed or when purified hRS1 protein was injected into hSGLT1 expressing oocytes (21, 24, 27). We showed that hRS1 protein is a coat protein of the TGN and that it blocks the release of SGLT1 containing vesicles (21, 30). In the present study we tried to identify domains of hRS1 that are involved in posttranscriptional inhibition of hSGLT1. We co-injected cRNAs encoding various fragments of hRS1 together with cRNA of hSGLT1 into oocytes, incubated them 3 days for expression, and measured the uptake of 50 μM [14C]AMG mediated by hSGLT1. Fig. 1a shows that AMG uptake was inhibited when various fragments of hRS1 were coexpressed. An inhibition by 40–50% was observed when cRNA of intact hRS1 or cRNA fragments encoding amino acids (aa) 1–251 or 251–617 of hRS1 were expressed together with hSGLT1. This suggested that the N-terminal and C-terminal domains of hRS1 can mediate inhibition of hSGLT1, and that the inhibition by both domains is not additive. Focusing on the C-terminal part of hRS1 we performed additional truncations. Whereas cRNA fragments encoding aa 326–617 or 407–617 showed similar inhibition compared with cRNA encoding total hRS1, the inhibition by a cRNA fragment encoding aa 534–617 was significantly smaller although still significant. Because aa 572–611 of hRS1 represent an ubiquitin-associated domain (43) that may mediate various ubiquitin-related effects, we concentrated further analysis on RS1 fragments that do not contain the ubiquitin-associated domain. Performing further truncations we were able to attribute an inhibitory effect to a cRNA fragment encoding aa 407–415 of hRS1 (Fig. 1a).

The coexpression experiments described above do not differentiate between posttranscriptional short-term RS1 effects on SGLT1 and long-term effects of RS1 that may involve endog-
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enous oocyte proteins that participate in the regulation of SGLT1. For this reason, we expressed hSGLT1 in oocytes, injected 75 pmol of hRS1-derived peptides and measured uptake of 50 μM [14C]AMG 60 min later. Assuming that oocytes contain an internal aqueous volume of about 0.4 μl (38), the intracellular concentrations of injected peptides was about 0.2 mM. In initial experiments acetylated peptides were used because they may be more stable and more soluble in the cytosol. Fig. 1b shows that acetylated nonapeptide QNEQCPQVSac comprising aa 407–415 of hRS1 inhibited SGLT1-expressed AMG uptake by about 50%, whereas the acetylated reversed control peptide SVQPCQENQac revealed no significant inhibition. Injection of acetylated hexapeptides derived from QNEQCPQVS that overlap by three amino acids showed the same inhibition as the nonapeptide. The same inhibition was also obtained after injection of acetylated tripeptide QC Pac (aa 410–412 of hRS1) representing the overlapping amino acids. In contrast, the acetylated reversed tripeptide PCQac was not effective. Identical degrees of inhibition were observed with both QC Pac and nonacetylated QC P.

We wondered whether the cysteine in QC P is effective by forming a disulfide bridge to an intracellular protein and whether the tripeptides QSP and QPP that occur in the sequence of hRS1 are also effective. In the experimental series shown Fig. 1c we investigated various acetylated or nonacetylated tripeptides in which cysteine in the middle position was replaced by other amino acids. Under the employed experimental condition using an estimated intracellular peptide concentration of about 0.2 mM, significant inhibition of AMG uptake was not only observed for QC Pac, QSPac, QC Pac, and QSP, but also for QTPac, QPPac, QC Pac, QLPac, and QEP. The data suggest that the middle position in QC P is not critical for function, however, it may change affinity. Interestingly, hRS1 contains two QSP tripeptides in the N-terminal part (aa 19–21 and 91–93) and QPP (aa 311–313) in the middle part of the sequence.

To investigate whether the decrease of hSGLT1-mediated AMG uptake by QC P and QSP is additive we injected hSGLT1 expressing oocytes with 75 pmol of QC Pac, 75 pmol of QSPac, or 75 pmol of QC Pac plus 75 pmol of QSPac, incubated the oocytes for 1 h, and measured the uptake of 50 μM [14C]AMG. AMG uptake was decreased by 34 ± 7% by QC Pac, 44 ± 7% by QSPac, and 38 ± 7% by QC Pac plus QSPac (2 independent experiments). The data suggest that QC P and QSP activate the same mechanism to decrease AMG transport.

FIGURE 1. Identification of tripeptides derived from hRS1 that inhibit AMG uptake expressed by SGLT1. a, coexpression of hSGLT1 and fragments of hRS1. 2.5 ng of hSGLT1 cRNA were injected in X. laevis oocytes either alone (control) or together with 7.5 ng of cRNA encoding total hRS1 (aa 1–617) or the indicated hRS1 fragments. Oocytes were incubated for 3 days and uptake of 50 μM [14C]AMG was measured. Uptake rates were normalized to parallel measurements in control oocytes from the same batch. b, short-term effects of peptides derived from hRS1 on AMG uptake expressed by hSGLT1. 2.5 ng of hSGLT1 cRNA were injected in X. laevis oocytes, the oocytes were incubated for 3 days, and 25 nl of K-Ori buffer (control) or 25 nl of K-Ori buffer containing 75 pmol of the indicated peptides were injected. Acetylated (ac) and nonacetylated peptides were injected. One hour later uptake of 50 μM [14C]AMG was measured. Uptake measurements were normalized to parallel performed control measurements. c, effects of QC P or tripeptides where cysteine in QC P was replaced by other amino acids. The experiments were performed as in b. Mean ± S.E. are indicated. The number of independent experiments is given in parentheses. **, p < 0.01 and ***, p < 0.001 for difference to control; ●●, p < 0.01 and ●●●, p < 0.001 for difference to coexpression of hSGLT1 and the small hRS1 fragment containing the ubiquitin-associated domain of hRS1 (UBA).
Inhibition of SGLT1 Expression by Tripeptides

QCP Reduces the Amount of Expressed hSGLT1 within the Plasma Membrane—We investigated whether QCP decreases SGLT1-mediated AMG uptake in the same way as has been proposed for total hRS1, namely by decreasing the amount of SGLT1 in the plasma membrane via inhibition of SGLT1 release from the TGN (21). We isolated oocyte plasma membranes from non-injected control oocytes, from oocytes expressing hSGLT1, and from oocytes expressing hSGLT1 that were injected with 75 pmol of QCP, and incubated for 1 h. Because the effect of QCP was shown to be glucose-dependent (see below and Ref. 21) we incubated the oocytes 15 min with 50 µM AMG before the plasma membranes were purified. Separation of plasma membranes from intracellular vesicles was achieved using colloidal silica that binds to the glyocalix of plasma membranes (41). We observed some variation of purified plasma membrane proteins between different oocyte batches, however, within individual oocyte batches total plasma membrane protein was neither increased significantly after expression of hSGLT1 nor decreased significantly when QCPac was injected into hSGLT1 expressing oocytes. The total protein content of isolated plasma membranes normalized to that of non-injected oocytes was 1.01 ± 0.05 in hSGLT1 expressing oocytes and 0.92 ± 0.04 in SGLT1 expressing oocytes that had been injected with QCPac (five independent experiments). Western blots stained with an antibody against hSGLT1 showed that QCPac decreased the amount of hSGLT1 in the plasma membrane by about 50% (Fig. 2a).

To determine whether QCP decreases the number of functional active SGLT1 molecules, we performed capacitance measurements. Transport of D-glucose or AMG by SGLT1 or binding of phlorizin to SGLT1 is correlated with a decrease of capacitance that is proportional to the number of functional SGLT1 molecules (44). The left panel of Fig. 2b shows an experiment in which inward current and capacitance at −50 mV were measured when SGLT1 expressing oocytes were superfused with 5 mM AMG or 100 µM phlorizin. AMG is transported by hSGLT1 together with sodium and induces both, inward current and decrease of capacitance. Phlorizin does not induce current because it is not transported, however, it decreases capacitance because it blocks potential-dependent charge movements within hSGLT1. Because superfusion with AMG alters the intracellular concentration of AMG that modulates the effect of QCP on hSGLT1 (see below) we measured the effect of QCPac on phlorizin-induced capacitance changes. The right panel of Fig. 2b shows experiments in which phlorizin-induced capacitance changes were measured in hSGLT1 expressing oocytes that had been injected with K-Ori buffer or with K-Ori buffer containing 75 pmol of QCPac and incubated for 1 h. QCPac decreased phlorizin-induced capacitance changes by about 50%. The data indicate that the short-term inhibition of AMG uptake observed after injection of QCPac is due to a decrease of the number of functional hSGLT1 molecules in the plasma membrane.

QCP Blocks the Exocytotic Pathway of SGLT1—We tested whether fusion of intracellular vesicles with the plasma membrane and release of vesicles from the TGN are required for inhibition of hSGLT1 by QCP. We expressed hSGLT1 in oocytes and injected K-Ori buffer or K-Ori buffer containing 75 pmol of QCPac, 2 ng of BTXB, 0.5 pmol of BFA, QCPac plus BTXB, or QCPac plus BFA. After a 1-h incubation we measured AMG uptake (Fig. 3). BTXB cleaves synaptofibrin located at the intracellular vesicles (45), whereas BFA inhibits guanosine nucleotide exchange factors that activate ADP-ribosylation factors, which regulate the assembly of coat complexes at the TGN and endosomes that are involved in protein sorting and release of vesicles (46). BTXB inhibited SGLT1-mediated AMG uptake by 60%. This indicates that BTXB was active in our experiments and that exocytosis participates significantly in short-term regulation of SGLT1. Importantly, QCPac did not inhibit hSGLT1-mediated AMG uptake in the presence of BTXB. Fig. 3 shows also that BFA inhibited hSGLT1-mediated AMG uptake by

![Figure 2](http://www.jbc.org/)

**FIGURE 2.** QCP decreases the amount of hSGLT1 protein in the plasma membrane. A, quantification of hSGLT1 protein in the plasma membrane. Plasma membranes were isolated from non-injected, oocytes expressing hSGLT1, and oocytes expressing hSGLT1 that were injected with 75 pmol of QCP and incubated for 1 h. The left panel shows a Western blot from a typical experiment that was stained with affinity purified antibody against hSGLT1. 2 ng of protein was applied to each lane. A densitometric quantification of five independent experiments is shown on the right panel. ***, p < 0.001.** B, effect of QCP on phlorizin-induced capacitance changes in oocytes expressing hSGLT1. Oocytes expressing hSGLT1 were superfused with Ori buffer and clamped to −50 mV. Inward currents and capacitance were measured when oocytes were superfused with Ori buffer containing 5 mM AMG or 100 µM phlorizin (left panel). Phlorizin-induced capacitance decrease in hSGLT1 expressing oocytes injected with K-Ori buffer (hSGLT1) or K-Ori buffer containing 75 pmol/oocyte of QCPac (hSGLT1, QCPac) is compared on the right. Mean ± S.E. of 9 oocytes from three independent experiments are compared. ***, p < 0.001.
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50%. This indicates that the release of SGLT1 containing vesicles from endosomes or the TGN participates in short-term regulation. In the presence of BFA no inhibition of AMG uptake by QCPac was detected. The data indicate that QCPac blocks the exocytotic pathway of SGLT1 that is critically involved in short-term regulation.

To investigate how long the inhibitory effect of QCP persists, we injected 75 pmol of QCPac into oocytes, incubated the oocytes between 1 and 9 h at room temperature, and measured the uptake of 50 μM [14C]AMG. The inhibitory effect of QCPac persisted throughout the entire investigated time period (data not shown).

QCP and QSP Are High Affinity Down-regulators of hSGLT1—

We measured uptake of 50 μM [14C]AMG in hSGLT1 expressing oocytes 1 h after injection of various concentrations of QCPac, QCP, QSPac, and QSP (Fig. 4). Assuming a distribution volume of 0.4 μl/oocyte (38) IC50 values of 2.0 ± 0.8 nM (n = 5) (QCPac), 2.1 ± 0.3 nM (n = 2) (QCP), 0.17 ± 0.03 nM (n = 2) (QSPac), and 0.16 ± 0.01 nM (n = 3) (QSP) were obtained. The data indicate high affinity inhibition of hSGLT1 by QCPac and QSP. The affinity of the tripeptides is not changed by N-terminal acetylation, QSP has a 10-fold higher affinity compared with QCP.

Down-regulation of hSGLT1 by QCP and QSP Is Mono-

saccharide-dependent—Previously we reported that inhibition of hSGLT1-mediated AMG uptake in oocytes observed after injection of purified hRS1 protein was prevented when the oocytes were preincubated with D-glucose (21). We tested whether the protective effects of monosaccharides can be demonstrated for the inhibition of hSGLT1-mediated AMG uptake by QCP or QSP. We expressed hSGLT1 in oocytes and injected 25 nl of K-Ori buffer with and without 75 pmol of QCPac (Fig. 5, a, b, and e) or QSP (Fig. 5, c and d) containing various amounts of mannitol, sorbitol, D-glucose, D-fructose, AMG, or 2-deoxyglucose. Oocytes were incubated for 1 h at room temperature and uptake of 1.75 μM [14C]AMG was measured. Fig. 5a shows control experiments where we assessed potential effects of intracellular polyalcohols and monosaccharides on hSGLT1-mediated AMG uptake in the absence of the inhibitory tripeptides. AMG uptake was not influenced by intracellular sorbitol, mannitol, D-glucose, AMG, and 2-deoxyglucose up to a concentration of about 60 mM, and by intracellular D-fructose concentrations of 0.025, 0.25, 2.5, and 62.5 mM. At variance AMG uptake was 30% increased by 25 mM intracellular D-fructose. Inhibition of AMG uptake by QCPac was not changed by various intracellular concentrations of mannitol and sorbitol excluding osmotic effects (Fig. 5b). Protective effects of metabolized monosaccharides (D-glucose, D-fructose, and 2-deoxyglucose) and of the non-metabolized monosaccharide AMG on the inhibition of AMG uptake by QCPac and QSP were observed. For example, inhibition of AMG uptake by QCPac or QSP was significantly protected when the oocytes contained 0.25–62.5 mM D-glucose or D-fructose (Fig. 5, b and c). The monosaccharides D-fructose, AMG, and 2-deoxyglucose exhibited biphasic effects on the inhibition of hSGLT1-mediated AMG uptake by QCPac and QSP (Fig. 5, b and d). Inhibition of AMG uptake by tripeptides was most strongly protected by 0.25 mM AMG or 2-deoxyglucose. We determined the half-maximal concentrations for the protective high affinity effects (PC50) of 2-deoxyglucose and AMG (Fig. 5e). Significantly different values of 11.9 ± 0.8 μM (2-deoxyglucose) and 53 ± 1.7 μM (AMG) (n = 3 each, p < 0.001) were obtained. For the high affinity protective effect of D-glucose on inhibition of AMG uptake by QSP, a PC50 value of 30 ± 6.2 μM (n = 3) was determined (Fig. 5c). The data indicate that a high affinity monosaccharide binding site is involved in modulation of QCP/QSP-mediated down-regulation of hSGLT1.

Down-regulation of hSGLT1 by QCP Is Independent of PKC—

We investigated whether the effect of QCP on hSGLT1-mediated AMG uptake is increased after stimulation of PKC as has been observed for the effect of total hRS1 protein (21). We injected K-Ori buffer, K-Ori buffer containing 75 pmol of QCPac, or K-Ori buffer containing 75 pmol of QCPac plus 0.5 pmol of dioctanoylglycerol (stimulator of PKC) into hSGLT1 expressing oocytes, incubated the oocytes for 60 min and measured uptake of 50 μM [14C]AMG. In the absence of QCPac, dioctanoylglycerol increased hSGLT1-mediated AMG significantly as described earlier (21, 27) (Fig. 6). Whereas dioctanoylglycerol increased the short-term inhibition of AMG uptake after injection of purified hRS1 protein (21), dioctanoylglycerol did not alter the inhibition of AMG uptake after injection of QCPac. The same result was obtained when PKC was stimulated by PMA. In absence of QCPac, hSGLT1-mediated AMG uptake was significantly increased when oocytes were incubated for 2 min with 1 μM PMA, however, inhibition of AMG uptake after injection of QCPac was not changed. The data indicate that at variance to hRS1 the short-term down-regulation of hSGLT1 by QCP is independent of PKC. This suggests that the down-regulation of hSGLT1 by hRS1 is mediated via QCP and/or QSP sites in hRS1 that are modulated by PKC-dependent phosphorylation in hRS1.
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QCP and QSP Are Substrates of the Peptide Transporter PepT1—We wanted to evaluate the possibility to decrease small intestinal absorption of D-glucose by down-regulation of hSGLT1 via orally applied QCP or QSP. Because the tripeptides act intracellularly, we tested whether the human H$_{h}$SGLT1 via orally applied QCP or QSP. Because the tripeptides are substrates of hSGLT1. Measuring hPepT1-mediated inward currents at different concentrations of QCPac and QSP, Michaelis-Menten type activation curves were obtained (see e.g. Fig. 7b). At $-60$ mV, $K_{m}$ values of $21.4 \pm 6.6$ and $4.2 \pm 0.2$ mM ($n = 6$, each) were obtained for QCPac and QSP, respectively.

To determine whether hSGLT1 expression can be down-regulated by extracellular application of QCP in cells, we expressed hSGLT1 either alone or together with hPepT1 in oocytes. We then incubated the oocytes for $1$ h in either Ori buffer adjusted to pH 6.0 (control) or in Ori buffer, pH 6.0, that contained 150 $\mu$M QCPac or 150 $\mu$M PCQac. After a brief wash in Ori buffer, pH 7.6, we measured uptake of 50 $\mu$M [${}^{14}$C]AMG (Fig. 7c). In oocytes expressing hSGLT1 alone AMG uptake was not altered after incubation with extracellular QCP. However, in oocytes expressing hSGLT1 plus hPepT1, extracellular QCP decreased hSGLT1-mediated AMG uptake by 40%. At variance, the control peptide PCQac that is also transported by hPepT1 had no effect.

Phlorizin-inhibitable AMG Uptake into Caco-2 Cells Is Down-regulated after Incubation with QCP and QSP—To explore whether the hRS1-derived tripeptides supplied with the food are capable to down-regulate hSGLT1 in human small intestine we investigated whether extracellular application of tripeptides QCP and QSP to confluent human colon carcinoma (Caco-2) cells inhibits AMG uptake by hSGLT1. At late confluence, Caco-2 cells exhibit similar morphological characteristics as differentiated small intestinal enterocytes (47). They express hSGLT1 and hPepT1 and mediate phlorizin-inhibitable uptake of glucose.

D-Glucose Absorption in Rat Small Intestine Is Decreased after Perfusion with QSP Containing Solution—To test the effect of QSP in intact small intestine, 25-cm long small intestinal segments of male rats were perfused for 1 h with DPBS adjusted to pH 6.5 in the absence or presence of 15 mM QSP (Fig. 9). Thereafter the segments were perfused for 20 min with DPBS, pH 7.4, containing 10 $\mu$M D-glucose, and for another 20 min with DPBS containing 50 $\mu$M D-glucose. During the perfu-
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In the present study we identified the tripeptides QCP and QSP in sequence of hRS1 that act as high affinity posttranscriptional inhibitors of the Na\(^+\)-D-glucose cotransporter hSGLT1. Similar to total hRS1 protein (21), QCP and QSP inhibit an exocytotic pathway of hSGLT1. We showed that QCP decreases the amount of hSGLT1 protein in the plasma membrane and that the effect of QCP on hSGLT1-mediated AMG uptake was abolished when the release of vesicles from the TGN was blocked by BFA or when fusion of vesicles with the plasma membrane was blocked by BTXB. Recently we observed that hRS1 is located at the TGN and dissociates from the TGN after application of BFA (30). We concluded that hRS1 is a coat protein of the TGN. The similarities between the observed posttranscriptional effects of hRS1 and the tripeptides indicate that QCP and/or QSP are part of a posttranscriptionally active domain(s) of hRS1. The tripeptides are supposed to bind to a high affinity binding site of a protein that interacts with the TGN. hRS1 contains two QSP motifs in the N-terminal part (aa 19–21 and 91–93) and one QCP motif in the middle part (aa 410–
412). Because the effects of QCP and QSP were not additive both tripeptides may interact with the same tripeptide binding site. However, it is also possible that two binding sites at the same protein or at two proteins of the same regulatory pathway are involved. Whether both QSP motives in the N-terminal domain of hRS1 are effective, and whether the amino acids between these two sites that include two consensus sequences for the binding of protein 14-3-3 (aa 13–36 and 80–85) (49) and two consensus sequences for PKC-dependent phosphorylation (serines 45 and 66), play a functional role, must be clarified by future experiments. Because endogenous and overexpressed RS1 protein in HEK 293 cells, Caco-2 cells, and LLC-PK1 cells is rapidly degraded, and we demonstrated inhibitory activity of small hRS1 fragments including tripeptides, it is possible that the posttranscriptional down-regulation of hSGLT1 by hRS1 is partially mediated by hRS1 fragments. However, the observation that the inhibition of hSGLT1 expression after injection of total hRS1 protein was increased when PKC was activated, whereas the inhibition by QCP was independent of PKC, suggests that the physiologically active domain of hRS1 contains a PKC site or is regulated by a distant PKC site in hRS1.

In the present study the previously described modulatory effect of AMG on posttranscriptional regulation by hRS1 (21) was further investigated. We observed similar effects of AMG, 2-deoxyglucose, d-glucose, and d-fructose on QCP- or QSP-

\[ \text{FIGURE 6. Analysis of the effect of PKC stimulation on hSGLT1-expressed AMG uptake in the absence and presence of QCP. hSGLT1 expressing oocytes were injected with 25 nl of K-Ori without and with 75 pmol of QCPac and/or 0.5 pmol of dioctanoylglycerol (DOG). After 1 h incubation at room temperature, uptake of 50 \mu M [\text{14C}]AMG was measured. In some experiments the oocytes were incubated with 1 \mu M PMA 2 min prior to uptake measurements. The number of independent experiments in each which 8–11 oocytes were analyzed is given in parentheses. **, } p < 0.01; ***,***, } p < 0.001. The data indicate that PKC stimulation does not alter the effect of QCP on hSGLT1.\]

\[ \text{FIGURE 7. Uptake of QCP and QSP by hPepT1 and down-regulation of hSGLT1 in oocytes expressing hPepT1 after addition of QCP to the bath. Oocytes were injected with cRNA of hPepT1 (a–c), cRNA of hSGLT1 (c) or cRNAs of hSGLT1 and hPepT1 (c) and incubated 3 days for expression. In } a \text{ and } b, \text{ oocytes were superfused with modified Barth’s solution, pH 6.5, clamped to 60 mV, and inward currents induced by glycylglutamin (GQ), acetylated QCP (QCPac), or nonacetylated QSP were measured. } a, \text{ current traces. } b, \text{ concentration dependence of QSP induced inward currents (I_{QSP}). Mean } \pm S.E. \text{ of six oocytes are shown that were normalized to maximal GQ-induced currents measured after superfusion with 5 mM GQ (I_{GQmax}). The Michaelis-Menten equation was fitted to the data. c, down-regulation of hSGLT1 after incubation of oocytes expressing hSGLT1 plus hPepT1 with QCP. Oocytes expressing hSGLT1 or hSGLT1 plus hPepT1 were incubated for 1 h at pH 6.5 in the absence of peptides (control), or in the presence of 5 mM acetylated QCP or acetylated PCQ. Oocytes were washed and uptake of 50 \mu M [\text{14C}]AMG was measured. Mean } \pm S.E. \text{ of three independent experiments are presented. **, } p < 0.01.\]

H. Koepsell and A. Filatova, unpublished data.

mediated down-regulation of hSGLT1. The monosaccharides attenuated the inhibition of hSGLT1 transport activity by the tripeptides. The observed effects of metabolized monosaccha-
rides (2-deoxyglucose, D-glucose, and D-fructose) and the non-metabolized monosaccharide AMG, indicate the involvement of a hexokinase independent pathway. This pathway is triggered by a monosaccharide side with a $K_d$ of 30 $\mu M$ for D-glucose. For 2-deoxyglucose a lower apparent $K_d$ value was determined, however, we did not distinguish whether this is due to accumulation of 2-deoxyglucose 6-phosphate. The detected high affinity monosaccharide binding site cannot be located on hRS1 itself because monosaccharide effects were observed with hRS1-derived tripeptides, and tripeptides are supposed to be too small to form a monosaccharide binding site. This site has a different sugar specificity than SGLT1-mediated monosaccharide transport because 2-deoxyglucose was effective and SGLT1 does not translocate 2-deoxyglucose or 2-deoxyglucose 6-phosphate (1). However, it has been shown recently that rabbit SGLT1 contains an additional monosaccharide binding site with a different sugar specificity than the transport site that interacts with 2-deoxyglucose (50).

Previous data suggested monosaccharide-dependent post-transcriptional regulation of SGLT1. One study with rat jejunum showed that $V_{\text{max}}$ of phlorizin-inhibitable D-glucose uptake was increased 30 min after incubation with 25 mM D-glucose (51). In this work the authors did not distinguish whether the SGLT1 protein in the plasma membrane or the turnover of SGLT1 was increased. In another study in which Caco-2 cells were preincubated for 1 h with 100 mM D-glucose, a 45% decrease of phlorizin-inhibitable AMG uptake was observed (20). The authors proposed that this down-regulation is due to increased endocytosis, however, no significant changes of the intracellular distribution of SGLT1 could be detected. In small intestine, monosaccharide-dependent regulation of SGLT1 has been also observed on the level of transcription (10, 18). In yeasts, monosaccharide-dependent post-transcriptional regulation of several hexose transporters has been described (52). Recently, it has been reported that the monosaccharide H$^+$-cotransporter VvHT1 in grape is post-

**FIGURE 8.** Down-regulation of phlorizin-inhibitable AMG uptake into Caco-2 cells after preincubation with QCP or QSP. Caco-2 cells were grown for 9 days after confluence, incubated for 1 h at pH 6.0 in the absence or presence of QCPac (a) or QSP (b), and uptake of 50 $\mu M$ $[^{14}C]$AMG was measured in the absence or presence of 0.5 mM phlorizin. Total AMG uptake (left panels) and phlorizin-inhibited AMG uptake (right panels) are indicated. Mean ± S.E. of five independent experiments are presented. **, $p < 0.01$; ***, $p < 0.001$.

**FIGURE 9.** Effect of QSP on the D-glucose absorption in rat small intestine. Small intestinal segments of male rats were first perfused with DPBS adjusted to pH 6.5 in the absence (open circles) and presence of 15 mM QSP (closed circles). Thereafter the segments were washed and perfused with DPBS (pH 7.4) containing 10 mM D-glucose and with DPBS containing 50 mM D-glucose. The glucose concentration in the effluents was measured in 2.5-min intervals. Mean ± S.E. of three control animals and four animals treated with QSP are shown. $p < 0.05$.

$^5$ M. Veyhl, A. Vernaleken, and H. Koepsell, unpublished data.
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transcriptionally regulated by the non-metabolized monosaccharide 3-O-methyl-D-glucose (53).

Do mammals need monosaccharide-dependent posttranscriptional and transcriptional regulation of SGLT1 in addition to regulation by enterohormones (14, 15, 54) and the autonomous nervous system (13)? Apparently mammals developed a highly complex regulatory network in which small intestinal sugar absorption steers the regulation of glucose uptake systems. Both, the glucose concentration in the small intestinal lumen and the activity of glucose uptake systems (54, 57). Both, the glucose concentration in the small intestinal lumen and the activity of glucose uptake systems (54, 57). Both, the glucose concentration in the small intestinal lumen and the activity of glucose uptake systems (54, 57).

Because SGLT1 in the brush-border membrane of enterocytes mediates the first step in glucose absorption, monosaccharide-dependent regulation at this step may be very effective. After high glucose loading of small intestine GLUT2 distributes from the basolateral membrane of the enterocytes to the brush-border membrane (2). This redistribution of GLUT2 is supposed to be triggered by intracellular glucose and is consequently dependent on the function of SGLT1. Considering the described functions of SGLT1, the physiological importance of monosaccharide-dependent posttranscriptional regulations of SGLT1 for short-term adaptations is obvious. In addition, monosaccharide-dependent transcriptional regulation of SGLT1 expression is important for long-term adaptation of glucose absorption to diet.

The identification of tripeptides that down-regulate the expression of hSGLT1 and are substrates of the H\(^+\)/peptide cotransporter PepT1 opens new possibilities for treatment of obesity and diabetes type 2. PepT1 is expressed in enterocytes of the small intestine epithelial cells (32) and we showed that in cells expressing PepT1, the intracellular concentrations of QCP or QSP were high enough to down-regulate SGLT1 when the tripeptides were added extracellularly. This was demonstrated in oocytes, in polarized Caco-2 cells that are widely used as a model for human enterocytes (47), and in isolated and perfused rat small intestine. These experiments showed that QCP or QSP added to the food may down-regulate small intestinal \(\text{D-glucose}\) and \(\text{D-galactose}\) absorption by 20–50%. In addition to reducing energy supply this is beneficial for the prevention and treatment of diabetes because it alleviates postprandial glycemnic excursions as has been shown for the \(\alpha\)-glucosidase inhibitors acarbose (60, 61). Glycemic peaks during diabetes are responsible for the desensitization of insulin receptors and cause vascular and renal complications. It has been shown that acarbose treatment of patients with impaired glucose tolerance was correlated with decreased incidence of diabetes type 2 (61). QSP and related compounds are supposed to have a similar beneficial effect but two advantages compared with acarbose. Two daily oral doses should be enough to obtain down-regulation of glucose absorption, and overdosage does not lead to side effects due to sugar malabsorption.

In future experiments we want to elucidate whether QSP or related compounds may also be useful to decrease postprandial glucose peaks in the blood via inhibition of renal glucose reabsorption. Several pharmaceutical companies focus on this strategy using analogs of phlorizin that are selective for hSGLT2 in comparison to hSGLT1 (62–64). SGLT2-specific inhibitors are preferred for two reasons. First, the bulk of filtrated glucose is reabsorbed by the low affinity transporter SGLT2 that is expressed in early proximal tubules, whereas the high affinity transporter SGLT1 in late proximal tubules mediates fine adjustment of glucose reabsorption (1). Second, human SGLT1 is highly expressed in heart (65, 66) and inhibitors of SGLT1 may impair energy supply of cardiac myocytes. Preliminary experiments showed that QCP and QSP also mediate short-term down-regulation of hSGLT2 and are also transported by the H\(^+\)/peptide cotransporter PepT2. Using a QSP-related compound that is absorbed in small intestine or applied intravenously, it may be possible to down-regulate SGLT1 and SGLT2 in the kidney. After glomerular filtration, such a compound will be taken up by proximal tubules via PepT1 and PepT2 (32). Because QSP-related compounds only act intracellularly, they should not down-regulate hSGLT1 in heart, where no H\(^+\)/peptide cotransporters are expressed (65, 66).

In summary, we identified tripeptides in hRS1 that mediate posttranscriptional down-regulation of hSGLT1 at the TGN. We indicated that the tripeptides activate an inhibitory pathway that is modulated by intracellular monosaccharides. These tripeptides and related compounds are potential drugs for treatment of obesity and diabetes type 2.

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Tripeptides of RS1 (RSC1A1) Inhibit a Monosaccharide-dependent Exocytotic Pathway of Na\(^+\)-d-Glucose Cotransporter SGLT1 with High Affinity

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