Desoxyrhaponticin (3,5-Dihydroxy-4’-methoxystilbene 3-O-β-D-glucoside) Inhibits Glucose Uptake in the Intestine and Kidney: In Vitro and in Vivo Studies

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ABSTRACT

Rhubarb extracts have been reported to improve oral glucose tolerance in diabetic animals. In the present study we have investigated the antidiabetic actions of desoxyrhaponticin, a major stilbene in rhubarb, as a glucose uptake inhibitor. Desoxyrhaponticin was demonstrated to inhibit glucose uptake in rabbit intestinal membrane vesicles as well as in rat everted gut sleeves, with IC₅₀ values of 148.3 and 30.9 µM, respectively. Kinetics studies revealed that desoxyrhaponticin is a competitive inhibitor of glucose uptake in both systems. Moreover, desoxyrhaponticin could reduce glucose uptake in the intestinal membrane vesicles of both normal and diabetic rats. In addition, glucose uptake in the renal membrane vesicles of both normal and diabetic rats was reduced by desoxyrhaponticin. Under the inhibition of desoxyrhaponticin, uptake of glucose in both the intestinal and renal membrane vesicles of the normal rats was no different from that of the diabetic rats. The IC₅₀ values of the uptake inhibition in the renal membrane vesicles of normal and diabetic rats were 118.8 and 115.7 µM, respectively. In a type 2 diabetic animal model in which rats have been treated with streptozotocin at the neonatal stage, postprandial hyperglycemia was significantly suppressed by oral administration of this compound (300 mg/kg b.wt.). These results suggest that desoxyrhaponticin is an agent that is potentially effective in controlling postprandial hyperglycemia in diabetes. The in vivo antidiabetic action of this compound can be explained, in part at least, by inhibition of glucose transport in the small intestine and inhibition of glucose reabsorption in the kidney.

Type 2 diabetes mellitus (DM) accounts for 90 to 95% of all cases of diabetes and has become a major health issue worldwide in recent years. The problem is getting increasingly more serious in both developed and developing countries with a rapidly expanding diabetic population that is getting younger, probably due to changes in dietary habits and exercise patterns in recent years. The increasing morbidity and mortality of diabetes complications have exerted tremendous pressure on the health care system. Several epidemiological studies have suggested that increased glycemic exposure, especially during postprandial hyperglycemia, is an independent risk factor of vascular diseases (Ceriello, 2005). Evidence is emerging that this association is also present in the prediabetic and nondiabetic states (Muntner et al., 2005). Apart from injectable insulin and its analogs, four distinct classes of oral hypoglycemic agents are currently available. These include agents that could stimulate insulin secretion (e.g., sulfonylureas), reduce hepatic glucose production (e.g., biguanides), delay carbohydrate digestion (e.g., α-glucosidase inhibitors), and improve insulin sensitivity (e.g., thiazolidinediones). Despite the understanding that normalization of hyperglycemia could prevent the majority of diabetes complications (UK Prospective Diabetes Study Group, 1998), the available treatment regimens cannot adequately normalize blood glucose levels in diabetic patients. Thus, there is a need for agents that can achieve better glycemic control.

Over the years, various medicinal plants and their extracts have been reported to be effective in the treatment of hyperglycemia and diabetes (Marles and Farnsworth, 1995). The
hypoglycemic actions of some of these phytochemical constituents have been evaluated and confirmed in animal models (Hwang et al., 2005; Vinson and Zhang, 2005), suggesting that natural products could serve as a source in the search for effective antidiabetic agents.

Rhubarb is a common herb used in traditional Chinese medicine. The medicinal components used consist of the underground parts (rhizome and root) of Rheum officinale Bail, Rheum tanguticum Maxim. ex Balf, and Rheum palmatum Linn. (Polygonaceae). Various biological actions have been reported for rhubarb including treatment of diarrhea (Oi et al., 2002), lowering of blood urea nitrogen (Yokozawa et al., 1991), and antibacterial (Park et al., 2002) and antiviral actions (Kim et al., 2001). The principal constituents of the official rhubarb are anthranoids including sennosides, rhein, rhaponticin, desoxyrhaponticin, and desoxyrhaponticin. Recent studies have also indicated the possible antidiabetic actions of the extracts and constituents of rhubarb (Babu et al., 2004; Choi et al., 2005, 2006).

Glucose absorption processes involve the transfer of sugar molecules across plasma membranes, which occurs via integral transport proteins. Two types of glucose transporters have been identified in higher organisms: Na\(^+\)-glucose cotransporters (SGLTs) and facilitated glucose transporters (GLUTs). The accepted model for glucose transport in the small intestine is that the sugar is actively taken up from the lumen into the cells across the brush-border membrane by SGLT1, a process driven by a Na\(^+\) electrochemical potential gradient (Meinild et al., 1998). Accumulated glucose then exits the cell across the basolateral membrane by a Na\(^+\)-dependent mechanism, involving GLUT2. For renal glucose transport, the sugar is actively taken up across the brush-border membrane of the proximal tubule by the low-affinity/high-capacity SGLT2 and GLUT2 in the early S1 segment and by the high-affinity/low-capacity SGLT1 and GLUT1 in the later portions of the proximal tubule (Chin et al., 1993; Marks et al., 2003). In both the intestine and kidney, the Na\(^+\)-dependent transport process is the dominant mechanism involved in the glucose uptake. In the present study, we have investigated the inhibitory actions of desoxyrhaponticin on Na\(^+\)-dependent glucose uptake in both the intestine and kidney.

**Materials and Methods**

**Materials.** Phlorizin (Fig. 1A), desoxyrhaponticin (Fig. 1B), and rhaponticin (Fig. 1C) were of the highest purity (99%) available from Sigma-Aldrich (St. Louis, MO). [1-\(^{2}H\)-]Glucose (20 Ci/mmol) and [1-\(^{3}H\)-]glucose (16.2 Ci/mmol) were also obtained from Sigma-Aldrich. d-[U-\(^{14}C\)]-glucose (230–370 Ci/mmol) was obtained from Amersham Biosciences (Buckinghamshire, UK). All other chemicals were of the highest analytical grade and were obtained from various commercial sources.

**Animals.** New Zealand White rabbits and Wistar rats were provided by the Laboratory Animal Services Center of the Chinese University of Hong Kong (Hong Kong, China). Animals were housed individually and allowed free access to standard laboratory chow and tap water unless otherwise specified. The animal rooms were controlled for temperature (25 ± 3°C), humidity (55 ± 5%), and light (12-h light/dark cycle).

**Induction of Diabetes in Rats.** A type 2 DM animal model in which rats were administered streptozotocin (STZ) was used (Blondel et al., 1989). Neonatal Wistar rats were injected with STZ (Sigma-Aldrich) i.p. (100 mg/kg in 50 mM citrate buffer, pH 4.5) on the first day of birth. This dosing is based on the fact that during the 1st week of life, the rats have the capacity to regenerate pancreatic \(\beta\)-cells and hence at the adult stage they would become mildly hyperglycemic (Portha et al., 1979). Control animals were injected with the buffer vehicle in parallel. All animals were weaned 21 days after birth, housed in stainless steel wire cages, and given standard laboratory chow and tap water ad libitum. The animals were used for the subsequent experiments at 12 weeks of age. A 2-h fasting plasma glucose level was determined for the 12-week-old rats. Rats in which the 2-h fasting plasma glucose concentrations were >140 mg/dl were defined as diabetic. Rats that were not successfully induced to become diabetic were excluded from the experiments.

**Plasma Glucose Level Determination.** The plasma glucose level was measured by an enzymatic-spectrophotometric glucose oxidase/peroxidase assay kit (BioSystem Development LLC, Middleton, WI). The assay was performed in 48-well plates. To 5 \(\mu\)l of plasma sample in each well, 1 ml of the assay reagent prewarmed at 37°C was added, and the mixture was incubated at 37°C for 5 min. The absorbance readings of the reaction mixtures from the plasma samples and the standards were measured at 500 nm against the blank on a plate reader.

**Preparation of Intestinal Brush-Border Membrane Vesicles.** Brush-border membrane vesicles were prepared from the small intestine of rabbits (Hauser et al., 1980) as well as from normal and diabetic rats (Balamurugan and Said, 2003) by the Mg\(^{2}\)+ precipitation method with some modifications. The small intestine of a freshly sacrificed, overnight-fasted New Zealand White rabbit was dissected from the pyloric sphincter to the ileocecal junction and immersed in ice-cold KCl solution (154 mM). The associated mesentery and fat were removed, and the contents of the intestine were gently...
squeezed out. The small intestine was then cut longitudinally and washed thoroughly in fresh ice-cold KCl solution. It was then mopped dry, weighed, snap-frozen, and stored in liquid nitrogen. Frozen small intestine was thawed in 200 ml of buffer 1 (10 mM D-mannitol and 2 mM HEPES-Tris, pH 7.1) and cut into small pieces. The epithelium was separated from the underlying mucosa by mechanical vibration for ~1 min using a Vibro-mixer (Vibromax, Racine, WI). The solution was then filtered under vacuum.

For rats, 10 normal or diabetic animals weighing 200 to 250 g each were fasted overnight and sacrificed by cervical dislocation. The small intestine was excised and washed with ice-cold saline, and the mucosa was scraped off with a glass slide. The intestinal mucosa was homogenized in buffer 1.

Brush-border membrane vesicles were obtained from the rabbit or rat intestinal homogenates according to the following procedures. The homogenate was first made up to 300 ml with buffer 1, and solid MgCl$_2$ was added to a final concentration of 10 mM. After being stirred for 20 min, the solution was centrifuged at 2400 g for 15 min at 4°C, and the supernatant obtained was then centrifuged at 30,000 g for 30 min at 4°C. The pellet was resuspended in buffer 2 (100 mM D-mannitol, 0.1 mM MgSO$_4$, and 2 mM HEPES-Tris, pH 7.4). This suspension was homogenized with a glass-Teflon homogenizer, and centrifuged at 30,000 g for 40 min at 4°C. The final pellet was resuspended in 1 ml of buffer 3 (300 mM D-mannitol, 0.1 mM MgSO$_4$, and 10 mM HEPES-Tris, pH 7.4), and the volume was measured and doubled with buffer 3. The solution was passed five times through a 28-gauge needle to generate the brush-border membrane vesicles. Finally, the preparation was divided into aliquots and kept frozen in liquid nitrogen until use. The activities of alkaline phosphatase (a marker enzyme for the brush-border membranes) and Na'/K'-ATPase were determined by the rapid filtration method (Hopfer et al., 1973). In brief, 20 µl of membrane vesicles (0.2–0.4 mg of protein) was added to 40 µl of 0.1 mM d-glucose in an incubation buffer containing 100 mM NaSCN and 100 mM mannitol in 10 mM HEPES-Tris (pH 7.4) with [1-3H]-d-glucose (~100,000 cpm) in a test tube at room temperature. Uptake was terminated after the requisite incubation time by addition of 1.5 ml of ice-cold stop-wash buffer containing 200 mM NaCl and 250 µM phlorizin in a 10 mM HEPES-Tris buffer, pH 7.4. The membrane vesicles were immediately filtered through a prewetted cellulose acetate-nitrate membrane filter (0.45 µm pore size; Millipore, Billerica, MA) and washed five times with 1.5 ml of ice-cold stop-wash buffer. The radioactivity on the membrane was measured on a Tri-Carb 2900TR liquid scintillation analyzer (PerkinElmer, Wellesley, MA) after addition of 3 ml of Hi-Safe II scintillation fluid from PerkinElmer.

D-Glucose Uptake by Everted Gut Sleeves. D-Glucose uptake by the rat everted gut system was measured according to the method established by Starck et al. (2000). Everted gut sleeves were prepared from the jejunal intestine of rats. Sleeves (~1.5 cm in length, two rats per experiment) were cut and randomized by swirling in cold oxygenated Krebs-bicarbonate buffer containing the following ingredients: 125 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl$_2$, 1.2 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$, and 20 mM NaHCO$_3$, pH 7.3 to 7.4 at 37°C, 290 mOsm. Pieces of the intestinal sleeves were mounted on a metal rod. The sleeve was secured over the grooves by ligatures, and excess tissues beyond the ligatures were cut away. Before the glucose uptake assay was performed, each everted sleeve was preincubated in a flat-bottomed water-jacketed container with 5 ml of oxygenated glucose-free buffer and stirred at 1200 rpm for 5 min at 37°C. It was then transferred to another container containing 4 ml of oxygenated 50 mM d-glucose buffer with [U-14C]glucose (~100,000 cpm) and [1-3H]-glucose (~100,000 cpm) for 2 min. The everted sleeve was immediately rinsed briefly in ice-cold buffer stirred at 1200 rpm for 20 s to terminate the uptake process. The rinsed sleeves were cut, weighed, and solubilized with 1 ml of Soluene-350 from PerkinElmer for liquid scintillation counting using the 3H/14C dual-label protocol on a Tri-Carb 2900TR liquid scintillation analyzer.

Carrier-mediated d-glucose uptake was calculated as the uptake of [U-14C]glucose, corrected both for passive glucose uptake and for glucose in the adherent fluid by subtracting the simultaneously measured uptake of the stereoisomer [1-3H]-glucose. The latter is not subject to carrier-mediated transport. Uptake was normalized to the wet weight of the tissue.

Preparation of Renal Brush-Border Membrane Vesicles. Renal cortical brush-border membrane vesicles were prepared from the kidneys of normal or diabetic rats by an established MgEGTA procedure (Biber et al., 1981). The whole procedure was carried out at 4°C. In brief, kidneys were removed from 10 rats and decapsulated before obtaining the renal cortex. The cortex slices were homogenized for 2 min in a buffer containing 300 mM D-mannitol, 5 mM EGTA, and 10 mM HEPES-Tris, pH 7.4, by a Polytron on setting 5. MgCl$_2$ was added to give a final concentration of 12 mM. After 20 min, the homogenate was centrifuged at 2400 g for 15 min. The supernatant was collected and centrifuged at 30,000 g for 30 min. Afterward, the pellet was then resuspended in a buffer containing 150 mM D-mannitol, 2.5 mM EGTA, and 5 mM HEPES-Tris, pH 7.4, and homogenized on a glass-Teflon homogenizer, and then treated at a final concentration of 12 mM MgCl$_2$ for 20 min. The homogenate was centrifuged at 2400 g for 15 min. The supernatant was collected and centrifuged at 30,000 g for 30 min. The final pellet was resuspended in buffer 3, and the pellet volume was measured and doubled with the same buffer. The solution was passed 5 times through a 28-gauge needle to generate the vesicles and then divided into aliquots and stored frozen in liquid nitrogen until use. The specific activities of alkaline phosphatase in the brush-border membrane vesicle preparations were determined by 9- to 12-fold compared with those of the initial homogenates. In contrast, the level of the Na'/K'-ATPase activity of the preparations did not increase.

Protein Assay. The protein concentrations of the brush-border membrane vesicle preparations were measured by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Oral Glucose Tolerance Test in Diabetic Rats. A type 2 DM rat model created by the neonatal administration of STZ as described previously was used. After 2 h of fasting of the 12-week-old diabetic rats, an oral glucose tolerance test (OGTT) was performed. Glucose (2 g/kg b.wt.) was orally administered to the diabetic rats as a 0.4 g/ml glucose solution. Desoxystrophanthin was administered to the animals (at 75, 150, and 300 mg/kg b.wt.) in a 50% PEG 400 solution just before the glucose loading. Animals in the untreated diabetic group were given the 50% PEG 400 solution as the solvent control. Blood samples were collected from the tip of the animals’ tails before the glucose administration and at different times afterwards up to 120 min. The plasma glucose level was measured by the method described above. The values of the total change in plasma glucose level (ΔG) from the basal level at time 0 during the course of the 120 min were calculated for both the untreated diabetic animals and the drug-treated animals.

Analysis of Data. The apparent kinetic parameters $K_m$ (Michaelis constant) and $K_i$ (inhibition constant) of glucose uptake by the rabbit brush-border membrane vesicles and rat everted gut sleeves were calculated according to the equation $K = K_m \left[ I / (I + K_{i}) - K_m \right]$, where $I$ is the concentration of the inhibitor and $K_{i}$ is the apparent $K_m$ in the presence of the inhibitor. All data are presented as mean values ± S.E.M. To determine statistical significance, the data were evaluated using either the unpaired Student’s t test or two-way
analysis of variance by the computer software Prism 4 from GraphPad Software Inc. (San Diego, CA), and a value of \( P < 0.05 \) was considered statistically significant. The calculation of the 50% inhibitory concentration (IC\(_{50}\)) was performed by a nonlinear subroutine of the software.

**Results**

**Desoxyrhaponticin Inhibits Glucose Uptake in Rabbit Small Intestine Brush-Border Membrane Vesicles.** D-Glucose transport in rabbit small intestine was determined by the Na\(^+\)-dependent uptake in the brush-border membrane vesicles. A time course study up to 3600 s is shown in Fig. 2. Glucose uptake into the intestinal membrane vesicles exhibited a typical "overshoot" phenomenon at short incubation times, reaching its peak value at 40 to 60 s. This demonstrated the functional integrity of the membrane preparations. An incubation time of 60 s was used in subsequent experiments.

Desoxyrhaponticin and phlorizin (a positive control for glucose uptake inhibition) were initially dissolved in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in the medium was 1% (v/v), at which no appreciable effect on glucose uptake was detected. As shown in Fig. 2, glucose uptake in the entire uptake profile was significantly decreased in the presence of desoxyrhaponticin. At 60 s, the value of the glucose uptake was reduced by almost 50% from 1166.7 ± 49.3 to 630.2 ± 6.3 pmol/mg protein. Phlorizin at the same concentration elicited a more potent inhibitory action on the glucose uptake. This initial study of the action of the glucose transport inhibitors on the entire uptake profile is important, as it indicates that the inhibition of glucose uptake in all subsequent assays performed at 60 s was not due to a delay in the uptake kinetics but rather to an actual decrease in the whole uptake profile.

Desoxyrhaponticin as well as phlorizin inhibited glucose uptake into the intestinal brush-border membrane vesicles in a concentration-dependent manner (Fig. 3). The IC\(_{50}\) values of desoxyrhaponticin and phlorizin were determined to be 148.3 and 26.9 \( \mu \)M, respectively. Phlorizin was approximately four times more potent than desoxyrhaponticin in this assay. Interestingly, rhaponticin, which is a structural analog of desoxyrhaponticin, is inactive toward glucose uptake in this assay, exhibiting only a small effect at 500 \( \mu \)M (data not shown).

**Desoxyrhaponticin Inhibits Glucose Uptake in Rat Everted Gut Sleeves.** The inhibitory action of desoxyrhaponticin on D-glucose uptake was also investigated using the rat everted gut sleeve technique. Na\(^+\)-dependent D-glucose uptake into rat everted sleeves was determined, and the data were normalized to the wet weight of the tissue. Everted gut sleeves were incubated in Krebs-bicarbonate buffer containing radioactively labeled glucose with or without the test compounds. As shown in Fig. 4, desoxyrhaponticin exhibited a concentration-dependent inhibition of glucose uptake in the everted intestinal sleeves. The IC\(_{50}\) values of desoxyrhaponticin and phlorizin were determined to be 30.9 and 1.9 \( \mu \)M, respectively. Although the inhibitory actions of desoxyrhaponticin on the rabbit intestinal brush-border membrane vesicle system and the rat everted gut system were comparable, the latter appeared to be much more sensitive toward the inhibitory action of phlorizin.

**Desoxyrhaponticin Is a Competitive Inhibitor of Glucose Uptake.** The kinetics of the glucose uptake inhibition in both the rabbit intestinal brush-border membrane vesicle system and the rat everted gut system were further evaluated by varying the D-glucose concentrations in the assay medium in the presence or absence of the inhibitor. The results were analyzed by Lineweaver-Burk transformation of the uptake kinetics data (Fig. 5, A and B). In the absence of the inhibitor, the \( K_m \) of the glucose uptake was

![Fig. 2. Inhibitory action of desoxyrhaponticin on glucose uptake into rabbit intestinal brush-border membrane vesicles. Glucose uptakes in the membrane vesicles were measured as described under Materials and Methods at room temperature in the absence (control, ○) or presence of desoxyrhaponticin (500 \( \mu \)M, ●) or phlorizin (500 \( \mu \)M, ■). Each point represents the mean value ± S.E.M from four separate membrane vesicle preparations and individual points were performed in triplicates in each experiment. When not given, S.E.M. bars are smaller than the symbol used. Ordinate units are picomoles of glucose uptake per milligram of protein of membrane vesicles. *, \( P < 0.001 \), significantly different from the respective control values in the absence of any uptake inhibitor.](image1)

![Fig. 3. Concentration-dependent inhibition of glucose uptake into rabbit intestinal brush-border membrane vesicles by desoxyrhaponticin. Glucose uptakes in the membrane vesicles were measured for 60 s as described under Materials and Methods at room temperature in the absence (control) or presence of different concentrations of desoxyrhaponticin (10–500 \( \mu \)M, ●) or phlorizin (10–500 \( \mu \)M, ■). Experimental values in the presence of inhibitors were expressed as a percentage of the control. Each point represents the mean value ± S.E.M from four separate membrane vesicle preparations. When not given, S.E.M. bars are smaller than the symbol used.](image2)
determined from the double reciprocal plot to be 0.09 ± 0.04 mM for the rabbit intestinal membrane vesicle and 14.9 ± 1.3 mM for the rat everted gut. Desoxyrhaponticin was demonstrated to exhibit competitive inhibition on glucose uptake both in the rabbit intestine and in the rat intestine. \( K_i \) values were calculated to be 96.2 ± 43.3 and 1.1 ± 0.6 \( \mu \)M, respectively, for the rabbit intestinal membrane vesicle system and the rat everted gut system.

**Desoxyrhaponticin Inhibits Glucose Transport in the Intestine and Kidney of Normal and Diabetic Rats.** Figure 6 shows the actions of desoxyrhaponticin on glucose uptake in brush-border membrane vesicles prepared from the intestine (Fig. 6A) and kidney cortex (Fig. 6B) of normal and diabetic rats. The glucose uptake activities in the diabetic rats were found to be significantly higher than those in the normal rats, both for the intestine and kidney. Desoxyrhaponticin elicited a significant reduction in glucose uptake in the intestinal membrane vesicles of both normal and diabetic rats. Likewise, glucose uptake in the renal membrane vesicles of both normal and diabetic rats was reduced significantly by desoxyrhaponticin. Under the inhibition of desoxyrhaponticin, the uptake of glucose in both the intestinal and renal membrane vesicles of the normal rats was no different from that in the diabetic rats.

Furthermore, the concentration-dependent inhibitory actions of desoxyrhaponticin on the \( \text{Na}^+ \)-dependent glucose uptake activities in membrane vesicles prepared from the kidney cortex of normal (Fig. 7A) and diabetic (Fig. 7B) rats were also investigated. The \( IC_{50} \) values of desoxyrhaponticin and phlorizin were found to be 118.8 and 2.0 mM, respectively, for the normal rats and 115.7 and 1.8 mM, respectively, for the diabetic rats.

**Desoxyrhaponticin Reduces Postprandial Hyperglycemia in Diabetic Rats in Vivo.** The results of the OGTT for the diabetic rats are shown in Fig. 8A. The diabetic rats exhibited fasting hyperglycemia (plasma glucose concentration >140 mg/dl). After oral glucose loading, the animals in the untreated diabetic group exhibited postprandial hyperglycemia (plasma glucose concentration >200 mg/dl). Compared with the untreated diabetic rats, no significant difference in the solvent control (PEG 400) group was observed. Animals in the desoxyrhaponticin treatment groups at 150 and 300 mg/kg had significantly lower plasma glucose concentrations than those in solvent control group at 40 and 60 min (Fig. 8A). The values of total change in plasma glucose (\( \Delta G \)) from the basal level during the 120-min experimental period after 300 mg/kg desoxyrhaponticin administration were significantly smaller than those for the solvent control group (Fig. 8B).

**Discussion**

The importance of postprandial glycemic control in the development of diabetic complications is widely accepted on...
the basis of many epidemiological studies. Postprandial hyperglycemia in DM induces oxidative stress generation and elicits vascular inflammation and platelet activation, thus increasing the risk of vascular complications (Ceriello, 2005).

It has been shown in rats with experimentally induced diabetes and in patients with type 2 diabetes that the capacity of the small intestine to absorb D-glucose increases. This is due to a combination of intestinal structural changes with a specific increase in the expression of the monosaccharide transporters SGLT1, GLUT5, and GLUT2 (Dyer et al., 1997). Large amounts of GLUT2 protein appeared in the apical membrane vesicles of diabetic rats (Kellett and Brot-Laroche, 2005). Recently, a new model of sugar absorption, the apical GLUT2 model, in diabetes has been proposed. Before a meal, there is little glucose in the lumen and GLUT2 is very low at the apical membrane. However after a meal, as the concentration of free glucose increases, initial transport across the apical membrane occurs through SGLT1, causing activation of protein kinase CβII. These events result in rapid activation of apical GLUT2 already in the membrane and further insertion of GLUT2 into the apical membrane from intracellular vesicles underlying the membrane. Apical GLUT2 subsequently becomes the major pathway of glucose absorption.

Control over the intestinal absorption of glucose thus constitutes an avenue of glycemic control in diabetes. SGLT1 and GLUT2, which exist not only in the intestinal epithelial

**Fig. 6.** Inhibitory actions of desoxyrhaponticin on D-glucose uptake into intestinal and renal brush-border membrane vesicles of normal and diabetic rats. Intestinal (A) or renal (B) brush-border membrane vesicles were prepared from both normal and diabetic rats, and D-glucose uptake measured as described under Materials and Methods. Intestinal (A) or renal (B) brush-border membrane vesicles were incubated for 1 min in an uptake medium containing [1-3H]D-glucose at room temperature in the absence or presence of desoxyrhaponticin (500 μM). Data presented are the mean values ± S.E.M. (n = 4) on separate membrane vesicle preparations, and experiments for individual points were performed in triplicates in each case. Ordinate units are picomoles of glucose uptake per minute per milligram of protein. *, P < 0.05; **, P < 0.01 when values of the diabetic rats are compared with the values in the normal rats.

**Fig. 7.** Concentration-dependent inhibition of glucose uptake into renal brush-border membrane vesicles of normal and diabetic rats. Normal (A) and diabetic (B) rat renal brush-border membrane vesicles were incubated for 1 min in an uptake medium containing [1-3H]D-glucose at room temperature in the absence (control) or presence of different concentrations of desoxyrhaponticin (50–1000 μM, △) or phlorizin (0.5–50 μM, ○). Experimental values in the presence of inhibitors were expressed as a percentage of the control. Data presented are the mean values ± S.E.M. (n = 4) on separate brush-border membrane vesicle preparations, and individual points were performed in triplicates in each case. When not given, S.E.M. bars are smaller than the symbol used.
hyperglycemic effects worthy of further pharmacological evaluation. On the other hand, another natural product, quercetin, was reported to be a specific noncompetitive inhibitor of GLUT2. When diabetic rats were administered glucose with quercetin, hyperglycemia was significantly decreased compared with administration of glucose alone (Song et al., 2002).

In our studies, desoxyrhaponticin was demonstrated to exhibit moderate inhibitory actions on glucose uptake both in rabbit intestinal brush-border membrane vesicles (Fig. 3) and in the rat everted gut (Fig. 4), the potencies of which were comparable with each other, being 148.3 and 30.9 μM, respectively. This characteristic of desoxyrhaponticin as a glucose uptake inhibitor is very different from that of phlorizin in which a much bigger difference in potency between the two intestinal glucose uptake systems was noted. The rat everted gut system appeared to be more sensitive toward the inhibitory action of phlorizin than the rabbit intestinal membrane vesicle system. Lineweaver-Burk analysis of the kinetics data gave glucose uptake \( K_m \) values of 0.09 ± 0.04 mM for the rabbit intestinal membrane vesicles and 14.9 ± 1.3 mM for the rat everted gut, indicating that the rat everted gut system has a lower affinity for glucose than the rabbit intestinal membrane vesicle system. Desoxyrhaponticin was demonstrated to exhibit competitive inhibition on glucose uptake in both systems. The \( K_i \) was calculated to be 96.2 ± 43.3 μM for the rabbit intestinal membrane vesicle system and 1.1 ± 0.6 μM for the rat everted gut system.

Previous studies have shown that the capacity of the small intestine to absorb glucose in diabetic rats increases, involving events at the brush-border membrane. In the present study, we have observed that glucose uptakes in both the intestinal and renal membrane vesicles of diabetic rats are significantly higher than those of the normal rats. Desoxyrhaponticin was able to reduce glucose uptake in the intestinal and renal membrane vesicles of both the normal and diabetic rats. Under the inhibition of desoxyrhaponticin, the uptake of glucose in both the intestinal and renal membrane vesicles of the normal rats was no different from that in the diabetic rats.

Renal glucose reabsorption is mediated by the SGLTs (You et al., 1995) and the basolateral GLUTs. It has been reported that the expression and activity of both SGLTs and GLUTs were increased in diabetic rats (Domínguez et al., 1994; Vestri et al., 2001). In our experiments, glucose uptake in the renal membrane vesicles of diabetic rats was significantly higher than that of normal rats. Desoxyrhaponticin exhibited moderate inhibitory actions on glucose transport in the renal membrane vesicles of both the normal and diabetic rats. The \( I_{50} \) values were 118.8 μM in normal rats and 115.7 μM in diabetic rats. Previous reports on the existence of SGLTs in the kidney, together with our finding that desoxyrhaponticin could in fact inhibit glucose uptake in the kidney seem to suggest that the renal glucose reabsorption route might constitute another target for glycemic control in diabetes, apart from the intestinal absorption route. However, how important a reduced renal glucose transport is to glycemic control in diabetes is not entirely clear. A reduced glucose transport in the kidney might actually be counterproductive to the well-being of diabetic animal because the renal changes induced by desoxyrhaponticin could in theory increase osmotic diuresis and therefore exacerbate dehydration and electro-
lyte depletion during hyperglycemia. Such possible adverse effects of the compound on the kidney should be further investigated.

The whole animal study showed that the diabetic rats exhibiting fasting hyperglycemia (plasma glucose concentration >140 mg/dl) and postprandial hyperglycemia (plasma glucose concentration >200 mg/dl). Oral administration of desoxyrhaponticin at 300 mg/kg significantly suppressed this elevation of plasma glucose after oral glucose loading. These in vivo results indicate that desoxyrhaponticin exhibits a definitive hypoglycemic activity in the diabetic rats loaded with oral glucose. Taken together with our in vitro observations, the in vivo action of this compound can be attributed, in part at least, to the reduced glucose uptake from the intestine. However, we cannot rule out other possible actions of the compound such as altered gastric emptying, which might also contribute to the reduced postprandial hyperglycemia observed in the OGTT studies. Further studies are necessary.

In addition, whether the observed in vitro inhibitory effects of desoxyrhaponticin on the kidney membrane may have contributed to the in vivo altered OGTT awaits further elucidation. Most plant flavonoids undergo structural modifications during their passage across the gut mucosa and/or through the liver. It would therefore be necessary to determine the plasma profile of desoxyrhaponticin and its metabolites to draw a more definitive conclusion on this point.

The present study demonstrates the inhibitory actions of desoxyrhaponticin on Na+-dependent glucose uptake in both the intestine and kidney. It would be useful to know whether inhibitory effects on other Na+-dependent transport processes (e.g., amino acid uptake) are also present. If so, this would have nutritional implications should the compound be used on a long-term basis. Desoxyrhaponticin has also been reported to exert inhibitory actions on yeast and mammalian α-glucosidases (Babu et al., 2004). Results of the present study indicate that desoxyrhaponticin exhibits inhibitory action on small intestinal glucose uptake and renal tubular glucose reabsorption, providing another mechanistic explanation for the in vivo hypoglycemic action of rhubarb. On the other hand, however, rhaponticin, which is very similar in structure to desoxyrhaponticin, is almost devoid of such inhibitory activities. Interestingly, rhaponticin was also reported not to be capable of inhibiting α-glucosamylase activity (Choi et al., 2006). This indicates that a subtle difference in structure between desoxyrhaponticin and rhaponticin (which has an additional hydroxyl group compared with desoxyrhaponticin) is critical for their huge differences in bioactivities. A detailed structure-function analysis is highly desirable to arrive at a compound that is more potent as an antiobiotic agent.

In summary, we have demonstrated that desoxyrhaponticin could decrease glucose uptake in rabbit intestinal membrane vesicles and in the rat everted gut through inhibition of the Na+-dependent glucose transport. The compound exhibits competitive inhibitory action on glucose uptake in both systems. In addition, desoxyrhaponticin could reduce glucose uptake in the intestinal and renal membrane vesicles of both normal and diabetic rats. A subsequent in vivo study revealed that desoxyrhaponticin could improve oral glucose tolerance in diabetic rats. These results suggest that desoxyrhaponticin could play important roles in improving glycemic control to prevent the progression of hyperglycemia and diabetes complications. In view of the nontoxic nature of this natural compound, further studies on its possible clinical application for glycemic control in diabetic patients are highly warranted.

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