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trans-Resveratrol and ε-viniferin decrease glucose absorption in porcine jejunum and ileum *in vitro*

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ABSTRACT

trans-Resveratrol and ε-viniferin are used as dietary supplements. They are reported to be supportive in preventing arteriosclerosis and diabetes and a previous study could demonstrate an inhibitory potential on sodium-dependent glucose transport (SGLT1) in oocytes und mouse intestinal everted rings (Schulze et al., 2012, Genes Nutr. 6, S61). The *in vitro* effects of *trans*-resveratrol and ε-viniferin on intestinal glucose uptake in the porcine small intestines (*Sus Scrofa*) have not yet been evaluated. It was hypothesized that *trans*-resveratrol/ε-viniferin may have an adverse effect on porcine intestinal sodium-dependent glucose uptake. The effects on electrogenic small intestinal glucose absorption and sodium-dependent ^{3}H-glucose uptake in brush border membrane vesicles (BBMV) were evaluated. Pieces of mucosa were mounted into Ussing chambers and were incubated with either *trans*-resveratrol (0.3 mmol/L), ε-viniferin (0.3 mmol/L), or ethanol. Sodium-dependent glucose absorption into BBMV was measured. ^{3}H-glucose uptake studies were performed using the same concentrations of the respective substances. SGLT1-mediated glucose absorption was approximately 3-fold higher in ileum compared to jejunum. After preincubation with trans-resveratrol and ε-viniferin, glucose-induced increases of short-circuit currents were significantly decreased. BBMV-studies revealed comparable results and glucose uptake was also significantly decreased. As the glucose transport/uptake was decreased after preincubation with either *trans*-resveratrol or ε-viniferin this active transport mechanism was directly influenced by inhibiting the SGLT1 transport system.

1. Introduction

trans-Resveratrol (3,5,4`-trihydroxy-*trans*-stilbene) and its dehydrodimer *trans*-ε-viniferin (Fig. 1) are polyphenols which are abundant in a number of plants including the grapevine. They act as phytoalexins synthesized by the plants in response to fungal and bacterial infections or stress (Hain et al., 1990; Soleas et al., 1997; Pervaiz, 2003). Measureable amounts of both substances are found in grapes and products thereof especially red wine. Resveratrol and ε-viniferin have been shown to offer a variety of potential health benefits (Vang et al., 2011) such as anti-inflammatory (Chen et al., 2010; Kang et al., 2010), antioxidant (Jang and Surh, 2001; Chen et al., 2004, 2009) and anticarcinogenic properties (Saiko et al., 2008; Bai et al., 2010; Zghonda et al., 2011). ε-viniferin is supposed to have a comparable or even higher biological activity than resveratrol (Zghonda et al., 2011). Surprisingly ε-viniferin has not been sufficiently studied despite the fact that its content in red wine or grapesvines are comparable to resveratrol levels (Landrault et al., 2002; Mazauric and Salmon, 2005; Vitrac et al., 2005).

Beneficial effects on blood glucose levels may be based on alterations in intestinal glucose absorption as there is some evidence from studies on the human sodium-dependent glucose transporter 1 (SGLT1) expressed in oocytes and mouse intestinal everted rings, that *trans*-resveratrol and ε-viniferin have the potential to inhibit intestinal sodium-dependent glucose transport (Schulze et al., 2012).

The SGLT1 is the major route for absorption of glucose and galactose across the luminal membrane of swine enterocytes (Kellett, 2001; Moran et al., 2010). Although many data on the effects of *trans*-resveratrol and ε-viniferin are available, their effects on intestinal glucose transport in the small intestines have not yet been identified. As both substances are able to inhibit sodium-dependent glucose transport in oocytes and mouse intestinal everted rings (Schulze et al., 2012), it was hypothesized that *trans*-resveratrol and ε-viniferin may also exert an inhibitory effect on intestinal sodium-dependent glucose uptake via the SGLT1 in porcine jejunum and ileum. Due to the high physiological similarities of morphology and function of the gastrointestinal tract between humans and pigs, experiments were performed using tissue material from the intestinal tract of pigs (Larsen et al.,

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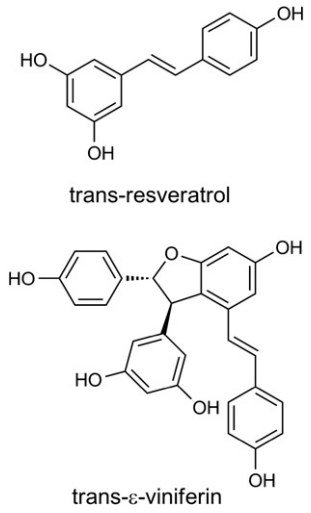

Fig. 1. Chemical structures of *trans*-resveratrol and its dehydrodimer ε-viniferin.

2002; Patterson et al., 2008; Strauss et al., 2008). The present study was conducted in order to monitor the effects of *trans*-resveratrol and ε-viniferin on electrogenic small intestinal glucose absorption using the Ussing–Chamber technique. Further characterization of sodium-dependent glucose uptake in the presence of *trans*-resveratrol and ε-viniferin was carried out by measuring ^{3}H-D-glucose uptake in isolated jejunal and ileal brush border membrane vesicles (BBMV).

2. Materials and methods

2.1. Materials

trans-Resveratrol (purity > 99%) was purchased from Sigma-Aldrich (Taufkirchen, Germany). ε-Vinferin (purity > 95%) was provided by Actichem SA (Montauban, France). Phlorizin (purity > 98%) was obtained from Sigma-Aldrich Chemicals (St. Louis, MO, USA). All other chemicals where purchased from Merck KgAA (Darmstadt, Germany), Serva GmbH (Heidelberg, Germany) and Sigma-Aldrich Chemicals (St.Louis, MO, USA) and were of 98% purity.

2.1. Animals

Eight healthy male and castrated crossbred growing pigs (*Sus scofa*), aged 6 to 8 weeks, were used for the experiments. Body weight before the start of the experiment ranged between 15 and 20 kg. Animals were kept in separate stables and were fed with a conventional standard chow (600 – 1000 g/d) twice a day; water was always available *ad libitum*. The standard chow contained 790 g/kg barley, 150 g/kg soybeans, 30 g/kg soy oil, 30 g/kg Proskana®80 (lysin-containing standard chow for pigs: 21.5% Ca, 8 % P, 5 % Na, 1 % Mg, additives/kg: 500 000 I.U. Vitamin A, 60 000 I.E. Vitamin D3, 1500 I.U. Vitamin E, 500 mg copper-2-sulfate, 38 % $CaCO_3$, 30 % $Ca(H_2PO_4)_2$, 12 % NaCl, 7 % $CaNaPO_4$, 5% sugar cane molasses, 1 % MgO). At the end of a three-week feeding period the animals were slaughtered by bleeding after stunning with captive bolt shot.

All procedures and handling of the pigs were in accordance with the German Animal Welfare Law.

2.2. Ussing chamber technique: electrogenic glucose transport studies

Within 2–3 min after slaughter the abdominal cavity was opened via incision of the ventral midline. The first 3.5 m of the small intestines aboral of the pylorus were discharged and the following 60 cm of mid jejunum were excised for Ussing chamber studies. In addition, 60 cm of ileum, nearby the ileocaecal fold, was taken. After withdrawal the intestinal segments were immediately rinsed with ice-cold physiological saline and stored in a standard buffer solution at 4 °C, being continuously aerated with carbogen (95% O_2, 5% CO_2) until further preparation. The standard buffer solution contained (mmol/L) 113.6 NaCl, 5.4 KCl, 0.2 HCl, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 21.0 $NaHCO_3$, 1.5 Na_2HPO_4 and 2.0 mannitol. After longitudinal incision of the mid jejunum and the ileum along the mesenteric fixation the mucosa was stripped of the underlying smooth muscle layers and subsequently separated into small pieces (1.5 × 1.5 cm). Thereafter, the tissue samples were manually mounted in Ussing chambers (Ussing, 1949a,b; Herrmann et al., 2012) with an exposed area of 1.13 cm^2. In addition to the standard buffer solution the buffer solution for the serosal side also contained (mmol/L) 10.0 glucose, 7.0 4-(−2hydroxylethyl)-piperazine-1-ethanesulfonic acid (HEPES) and 6.0 Na-gluconate. The mucosal buffer solution additively contained (mmol/L) 20.0 HEPES and 6.0 NaOH. The buffer solutions provided an osmolarity of 296 mosm/L and a pH value of 7.4 when aerated with carbogen at 37 °C. Indomethacin (10 μmol/L) was added to all buffer solutions to prevent a potential effect by endogenous prostaglandin production on ion-transport processes (Smith et al., 1981; Clarke and Argenzio, 1990).

From each pig and respective intestinal segment (mid jejunum, ileum) one Ussing chamber was equipped and connected to circulation reservoirs containing 10 mL buffer solution on each side at 37 °C (serosal and mucosal side of tissue sample), these being continuously aerated with 5% CO_2 in O_2 which maintained the pH level at 7.4.

Tissues were mounted into Ussing chambers. After 40 min of equilibration they were preincubated with 20 μL mucosal *trans*-resveratrol (final concentration: 0.3 mmol/L), ε-viniferin (final concentration: 0.3 mmol/L) or ethanol (EtOH) as a solvent control. The Ussing chambers were connected to a computer-controlled voltage clamp device (K. Mußler, Aachen, Germany). The experiments were performed under short-circuit current conditions. Short-circuit currents (I_{sc}) were recorded continuously throughout the whole experiment. Sodium-dependent glucose absorption was measured electrophysiologically by respective increases in short-circuit currents (ΔI_{sc}, $\mu eq^{*}h^{-1*}cm^{-2}$) after cumulative addition of glucose (mucosal: 0.5, 1, 2, 4 and 10 mmol/L; at 15 min intervals) (Fig. 2) in each chamber 30 min after preincubation with the respective active agent.

2.3. Preparation of BBMV

Tissue samples for the preparation of BBMV were taken from the mid jejunum and ileum (same anatomical location as for Ussing chamber studies). After rinsing with ice-cold physiological saline, samples were frozen in liquid nitrogen and stored at −80 °C until further use.

BBMV were isolated using a method based on Mg^{2+}-EGTA precipitation and differential centrifugation as previously described (Schroder and Breves, 1996). Resulting vesicle suspension was frozen in liquid nitrogen and stored at −80 °C until further use. Additionally, samples of the whole tissue homogenates and the vesicle suspensions were stored at −20 °C to determine of marker enzyme activities in order to assess vesicle purity and brush border enrichment. Therefore, specific activities of the apically located enzyme alkaline phosphatase (AP) and the basolateral marker Na^{+}/K^{+}-ATPase were measured using photometric standard methods (Ohmori, 1937; Mircheff and Wright, 1976). Protein concentration was determined according to Bradford 76 using Bio-Rad Protein Assay (Bio-Rad, München, Germany). The relative brush border enrichment was calculated as the ratio of the enrichment of marker enzymes in the vesicle suspension compared to the whole homogenate.

2.4. ^{3}H-D-glucose uptake into BBMV

Sodium-dependent D-glucose uptake into BBMV at a fixed D-glucose concentration of 1.0 mmol/L was studied by means of the rapid filtration