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Expression of sweet receptor components in equine small intestine: relevance to intestinal glucose transport

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Daly K, Al-Rammahi M, Arora DK, Moran AW, Proudman CJ, Ninomiya Y, Shirazi-Beechey SP. Expression of sweet receptor components in equine small intestine: relevance to intestinal glucose transport. *Am J Physiol Regul Integr Comp Physiol* 303: R199–R208, 2012. First published May 2, 2012; doi:10.1152/ajpregu.00031.2012.—The heteromeric sweet taste receptor T1R2-T1R3 is expressed on the luminal membrane of certain populations of enteroendocrine cells. Sensing of sugars and other sweet compounds by this receptor activates a pathway in enteroendocrine cells, resulting in secretion of a number of gut hormones, including glucagon-like peptide 2 (GLP-2). This subsequently leads to upregulation in the expression of intestinal Na^+/glucose cotransporter, SGLT1, and increased intestinal glucose absorption. On the basis of the current information available on the horse genome sequence, it has been proposed that the gene for T1R2 (*Tas1R2*) is absent in the horse. We show here, however, that horses express both the mRNA and protein for T1R2. Equine T1R2 is most closely homologous to that in the pig and the cow. T1R2 protein, along with T1R3, α-gustducin, and GLP-2 proteins are coexpressed in equine intestinal endocrine cells. Intravenous administration of GLP-2, in rats and pigs, leads to an increase in the expression of SGLT1 in absorptive enterocytes and enhancement in blood glucose concentrations. GLP-2 receptor is expressed in enteric neurons, excluding the direct effect of GLP-2 on enterocytes. However, electric stimulation of enteric neurons generates a neural response leading to SGLT1 upregulation, suggesting that sugar in the intestine activates a reflex increase in the functional expression of SGLT1. Horses possess the ability to upregulate SGLT1 expression in response to increased dietary carbohydrates, and to enhance the capacity of the gut to absorb glucose. The gut sweet receptor provides an accessible target for manipulating the equine gut to absorb glucose (and water), allowing greater energy uptake and hydration for hard-working horses.

sweet taste receptor

GLUCOSE IS TRANSPORTED ACROSS the intestinal brush border membrane by SGLT1 (41). This also activates water absorption, the basis for oral rehydration therapy (22). SGLT1 is the major route for the transport of glucose from the lumen of the intestine into absorptive enterocytes (12, 31). Thus, regulation of this protein is important for the provision of glucose to the body and avoidance of intestinal malabsorption. Expression of intestinal SGLT1 and the capacity of the gut to absorb glucose is upregulated in response to increased dietary carbohydrates in a range of species studied (16, 19, 31, 42, 45). This regulation is achieved through a signaling pathway initiated by activation of the gut sweet receptor, a heteromeric combination of taste receptor 1 family subunits, T1R2 and T1R3 (15, 30). The receptor functions in association with the guanine nucleotide-binding protein (G protein), gustducin (13, 30).

We have shown that T1R2, T1R3, and the α-subunit of gustducin are coexpressed in endocrine cells of the small intestine in a range of species (3, 30, 32). Deletion of either the T1R3 (*Tas1R3*) or α-gustducin (*GNAT3*) genes abolishes the ability of mouse intestine to increase SGLT1 expression in response to dietary carbohydrates or artificial sweeteners (13, 30). Further experimental evidence suggests that luminal sugars, via the sweet receptor expressed in enteroendocrine cells, stimulate secretion of gut hormones, such as glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide 1 (GLP-1), and GLP-2 (23, 30). In a number of species, intravenous administration of GLP-2 has been shown to increase SGLT1 expression in absorptive enterocytes (8, 9, 34, 39). However, GLP-2 receptor is expressed in enteric neurons and not on the basal membrane domain of absorptive enterocytes (2, 4), excluding the direct effect of GLP-2 on enterocytes. However, electric stimulation of enteric neurons generates a neural response leading to SGLT1 upregulation in absorptive enterocytes (43). Furthermore, perfusion of ileum with glucose increases Na^+-dependent glucose transport in the jejunum (11), suggesting that sugar in the intestine stimulates a reflex increase in the functional expression of SGLT1.

It has been demonstrated that glucose absorbed in the small intestine provides a substantial proportion of the horse's energy requirement (1, 37). This is particularly important when demand for glucose increases, such as during strenuous exercise, pregnancy, or lactation (1). We have previously shown that the major route for the absorption of glucose in the equine small intestine is via the SGLT1 (12). We cloned and sequenced the cDNA encoding for equine SGLT1, determined the amino acid sequence and produced specific antibodies (14). We further showed that, in horses maintained on a grain (starch)-based diet, there is an enhancement in the expression of intestinal SGLT1 compared with those fed a grass-based diet, indicating

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that the horse has the ability to enhance its intestinal capacity to absorb increased dietary carbohydrates (12).

However, until now, there has been little information on the equine intestinal sweet receptor or downstream signal transduction pathways. The major aim of this study was to assess whether the equine intestine expresses T1R2, T1R3, and key signaling elements required for SGLT1 upregulation. In the light of a report suggesting that the *Tas1R2* gene is absent from the horse genome (50), we aimed to investigate the validity of this proposal by cloning and sequencing cDNA encoding for equine T1R2. Horses express T1R3 and the amino acid sequence of equine T1R3 shows close homology to that in the pig and the cow. The amino acid sequence of cow T1R2 was deduced from the bovine genome; however, the sequence of pig T1R2, until now, has been unknown. To determine whether the observed homology between horse, pig, and cow T1R3 also exists for T1R2, it was required to clone and sequence pig T1R2.

The involvement of gut-expressed sweet taste receptor components in sensing dietary glucose in the intestine provides an attractive target for manipulating the capacity of the equine gut to absorb glucose (and water) with the attendant promise of enhancing energy uptake and hydration for hard-working horses.

MATERIALS AND METHODS

Animals and Tissues

Horse. Intestinal tissues were collected from eight adult horses (7- to 12-year-old mares and geldings of various breeds) euthanized at the University of Liverpool Philip Leverhulme Equine Hospital for reasons unrelated to gastrointestinal function, mainly due to limb injuries. The animals used were all client-owned horses undergoing euthanasia and, hence, outside the scope of Animals (Scientific Procedures) Act 1986. The samples were obtained with the informed consent of their owners, in accordance with the University of Liverpool policies. The intestinal tissue samples were removed promptly after euthanasia, sections of small intestine ~20 cm in length were removed from the proximal (30 cm distal to the pylorus), mid (half way along the small intestine), and distal (30 cm proximal to the ileocaecal junction) intestine. Sections were opened longitudinally, rinsed in ice-cold 0.9% (wt/vol) NaCl, and blotted with paper towel to remove excess mucus. One- to two-centimeter tissue samples were fixed for 4 h in 4% (wt/vol) paraformaldehyde-PBS and wax embedded before sectioning on a cryostat (Bright Instrument) for histological examination and immunohistochemical studies. Histological examinations revealed that tissues were intact with epithelial cells attached.

Lingual epithelial tissues taken from the tongue of the same horses were wrapped in aluminum foil, immediately frozen in liquid nitrogen and subsequently stored at −80°C until used for RNA extraction.

Pig. Lingual epithelial tissues were also taken from the tongue of healthy 41-day-old weaned piglets, killed by an intravenous injection of pentobarbital sodium (Pentoject; AnimalCare) to the cranial vena cava (in line with U.K. Home Office schedule 1 regulations) (31). The tissues were wrapped in aluminum foil, immediately frozen in liquid nitrogen, and subsequently stored at −80°C until used for RNA extraction.

Cat. Intestinal tissues were provided by the School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA (all procedures were approved by the Ethics Committee, University of Pennsylvania). Tissues were removed from healthy adult cats, used for research purposes at the Monell Chemical Senses Center and School of Veterinary Medicine, University of Pennsylvania, as described previously (3). Animals were euthanized by intravenous injection of pentobarbital sodium. The entire small intestine was removed immediately and opened longitudinally, rinsed in ice-cold 0.9% (wt/vol) NaCl, and blotted with paper towel. One- to two-centimeter pieces of mid-small intestine were excised and fixed for 4 h in 4% (wt/vol) paraformaldehyde-PBS, then placed in 20% (wt/vol) sucrose in PBS overnight. The samples were sent to the UK laboratory maintained at 4°C. Subsequently, tissue samples were gelatin-embedded and frozen in liquid N_2-cooled isopentane before sectioning on a cryostat (Bright Instrument) for histological examination and immunohistochemical studies. Histological examinations revealed that tissues were intact with epithelial cells attached.

Mouse. Intestinal tissues from horses euthanized for conditions such as limb injury, as used in this study, are normally removed as emergency cases and are either frozen or fixed immediately. Therefore, freshly removed mouse intestinal tissue was used for functional assays due to ease of availability. Small intestinal tissues were removed from male and female C57BL/6 mice immediately following euthanasia by cervical dislocation (UK Home Office Schedule 1 regulations). The tissue was opened longitudinally, and the serosa was removed by gentle scraping of the serosal side of the small intestine with a scalpel. Hematoxylin-and-eosin staining confirmed the removal of the serosa with minimal damage to the circular muscle layer. Sections of small intestine (2 cm) were used immediately for measurement of GLP-1 and GLP-2 secretion.

Cloning of Equine and Porcine T1R2 mRNA From Lingual Epithelia

RNA was extracted from equine and porcine lingual epithelia (RNeasy Mini Kit; Qiagen), according to the manufacturer's instructions. Single-stranded cDNA was produced using purified RNA as a template, oligo$(dT)_{20}$ primers, and Superscript III reverse transcriptase (Life Technologies). PCR was employed to produce amplicons of equine and porcine T1R2 cDNA, using consensus primers for T1R2 (based on known T1R2 mRNA sequences of cow, dog, human, and mouse), a proofreading DNA polymerase (Velocity; Bioline), and cDNA as template. These amplicons were then cloned into pGEM-T Easy Vector (Promega) and custom sequenced (Source BioScience). Sequence alignments were performed using commercial software (Vector NTi; Life Technologies). Phylograms were constructed by neighbor-joining analysis (38) of distance matrices generated using the PROTDIST program (Jones-Taylor-Thornton similarity model) (24), as part of the phylogenetic inference package, PHYLIP (18).

Immunohistochemistry

Slides, containing 8-μm-thick, wax-embedded serial sections of equine small intestinal tissue, were initially dewaxed in 100% xylene for 3 × 10 min, as described previously (12). Slides were then washed in 100% ethanol for 2 × 10 min and allowed to air dry. Frozen sections of cat small intestine, 8-μm thick, were thaw-mounted onto polylysine-coated slides and washed for 5 × 5 min in PBS. Sections were then circled with ImmEdge Hydrophobic Pen (Vector Laboratories), washed for 2 × 5 min in 70% ethanol before rehydration in double-distilled water for 2 × 5 min. Slides were then immersed in antigen retrieval buffer (10 mmol/l Tris/HCl [pH 10.0]) and autoclaved for 2 × 11 min at 126°C (Series 2100, Prestige Medical). Subsequently, slides were allowed to cool in antigen retrieval buffer at room temperature for 30 min before being washed for 3 × 5 min in PBS. Nonspecific antibody binding sites were blocked by incubating the tissue sections for 1 h in the appropriate blocking solution in a humidified chamber at room temperature. The following blocking solutions were used for procedures using antibodies to *1*) T1R2, T1R3, and α-gustducin: 5% (wt/vol) sucrose, 3% (wt/vol) BSA, 0.1% (wt/vol) NaN_3, and 2% (vol/vol) donkey serum in PBS; *2*) GLP-2: 3% (wt/vol) BSA, 0.1% (wt/vol) NaN_3 and 2% (vol/vol) donkey serum in PBS, and *3*) SGLT1 and ChrA: 10% (vol/vol) donkey serum in PBS. Sections were then incubated overnight at 4°C with primary polyclonal antibodies to T1R2 (H-90, raised in rabbit; T-20, raised in goat) (Santa Cruz Biotechnology), diluted 1:250; T1R3 (N-20, raised in goat; H-145, raised in rabbit) (Santa Cruz Biotechnology), diluted

1:250; α-gustducin (raised in rabbit) (a gift from Dr. R. F. Margolskee, Monell Chemical Senses Center, Philadelphia, PA), diluted 1:300; GLP-2 (C-20 raised in goat) (Santa Cruz Biotechnology), diluted 1:100; SGLT1 (custom synthesis, raised in rabbit), diluted 1:200; and ChrA (C-20, raised in goat) (Santa Cruz Biotechnology), diluted 1:100. For double-immunofluorescent staining, sections were incubated at 4°C overnight with two primary antibodies (raised in different species) without changing the final dilutions. Slides were then washed in PBS for 5 × 5 min and stained for 1 h at room temperature using a 1:500 dilution of either Cy3 or FITC-conjugated anti-rabbit/anti-goat/anti-mouse IgG (Jackson Healthcare). The composition of the buffer containing antibodies (primary or secondary) was 2.5% (vol/vol) donkey serum, 0.25% (wt/vol) NaN_3, 0.2% (vol/vol) Triton X-100 in PBS. Finally, slides were washed with PBS for 5 × 5 min and mounted in Vectashield hard set mounting media with DAPI (Vector Laboratories). Immunofluorescent labeling was visualized using an epifluorescence microscope (Nikon), and images were captured with a Hamamatsu digital camera (C4742–96-12G04; Hamamatsu Photonics). Images from double-immunofluorescent labeling were merged using Imaging Products Laboratory imaging software (BioVision Technologies). Cat intestinal tissue that does not express T1R2 protein (3) was used as the control to assess the specificity of T1R2 primary antibody binding to equine T1R2 protein. In addition, omission of primary antibodies was also used as a further control.

Measurement of GLP-1 and GLP-2 Secretion From Mouse Small Intestine

Freshly removed sections of mouse small intestine (2 cm) were incubated at 37°C in incubation media [Dulbecco's modified Eagle's medium (5.55 mmol/l glucose) (Sigma Aldrich), 10% (vol/vol) FBS, 2 mmol/l L-glutamine, 20-μl/ml DPPIV inhibitor (Catalog no. DPP4; Millipore)] supplemented with various concentrations of test reagents (glucose, sucralose, and gurmarin) for 1 h as indicated in figure legends. Control tissues were maintained simultaneously in incubation media in the absence or presence of gurmarin. After 1 h, incubation media were collected, centrifuged to remove cell debris, and stored at −80°C. GLP-1 and GLP-2 secretion was measured using commercially available enzyme immunoassay (EIA) kits (GLP-1: Millipore; GLP-2: Phoenix Pharmaceuticals), according to the manufacturer's instructions. Standard curves were constructed using GraphPad Prism 5 (GraphPad Software).

Statistical Analysis

Data in Fig. 6 are presented as means ± SE. Significance of differences was determined using Student's *t*-test (GraphPad Prism 5, GraphPad Software). Results were considered significant if $P < 0.05$. *P* values were confirmed by one-way repeated-measures ANOVA with Bonferroni's multiple-comparison test.

RESULTS

Cloning of T1R2 mRNA From Equine and Porcine Lingual Epithelia

PCR amplicons, produced from RNA extracted from equine lingual epithelium, were cloned and sequenced. A resulting 860-base pair (bp) sequence was screened against the National Center for Biotechnology Information (NCBI) nonredundant nucleotide database, via BlastN (49), identifying the cloned sequence as being homologous to T1R2 in many other mammalian species. The cloned mRNA fragment was translated to produce a sequence of 286 amino acids (corresponding to residues 521–806 of human T1R2). These 286 amino acid residues were then aligned using commercial software (Vector NTi; Life Technologies) to the corresponding regions of cow, dog, human, pig, and mouse T1R2 (Fig. 1). Phylogenetic analysis was performed to construct a radial phylogram depicting the relationship of equine T1R2 to related homologs in various other mammalian species for which sequence information is available (Fig. 2). Because of the lack of sequence information available for T1R2 in pig, PCR amplicons produced from RNA extracted from porcine lingual epithelium were also cloned and sequenced. Screening the complete porcine mRNA sequence against the NCBI nonredundant nucleotide database, via BlastN (49), identified the cloned sequence as being homologous to T1R2 in other species, closely related to cow and horse T1R2 (Fig. 2).

The NCBI accession numbers for the mRNA sequences of equine and porcine T1R2 are JQ424917 and JQ763389, respectively.

Validation of T1R2 Antibody Specificity Using a Genetic Approach

The primary antibody used in this study to identify equine T1R2 (H-90, Santa Cruz Biotechnology) is raised against a peptide sequence with high homology (66–74% amino acid identity) in a range of species. We have shown that this antibody specifically reacts with T1R2 protein expressed in the enteroendocrine cells of human, mouse, pig, and dog (3, 30, 32). To determine whether this antibody also specifically binds to equine T1R2 protein, we used sections of cat intestinal tissue, a naturally occurring "T1R2 knockout" model tissue (3, 27) as control. This method of assessment, i.e., using a genetic approach, is superior to using preabsorption controls (7). As observed in Fig. 3, the antibody reacts with a protein expressed in individual flask-shaped cells within the equine epithelial cell layer (Fig. 3, *A* and *B*), with a similar pattern of expression seen in the intestine of other species (3, 30, 32). In contrast, the antibody does not bind to any proteins in cat intestine (Fig. 3, *D* and *E*). The antibody to T1R3, however, reacts with proteins expressed in a subpopulation of cells in both cat and horse intestine (Fig. 3, *C* and *F*).

T1R2, T1R3, α-Gustducin, and GLP-2 Are Coexpressed in Equine Enteroendocrine Cells

Serial sections of equine small intestinal tissues were probed with primary antibodies to T1R2, T1R3, α-gustducin, and GLP-2. To determine any colocalization, images were merged (Fig. 4, *C*, *F*, *I*, and *L*). Horse intestine expresses α-gustducin and GLP-2 in a subset of cells (Fig. 4, *E* and *H*). Furthermore, T1R2, T1R3, α-gustducin, and GLP-2 are coexpressed with chromogranin A (ChrA), a classical marker of endocrine cells (coexpression of T1R2 and ChrA is shown in Fig. 4*L*). We have observed a similar colocalization pattern for T1R3 with T1R2, α-gustducin, GLP-2, and ChrA. T1R2 and T1R3 are also coexpressed with GLP-1 and GIP (data not shown). Patterns of expression of T1R2-T1R3 and α-gustducin were similar along the longitudinal axis of the small intestine, with slightly higher expression in proximal compared with distal regions (data not shown).

Expression of SGLT1 and T1R2

To determine the association between SGLT1 and T1R2 proteins in equine intestine, dual-immunofluorescent labeling was employed using antibodies to SGLT1 and T1R2. As seen

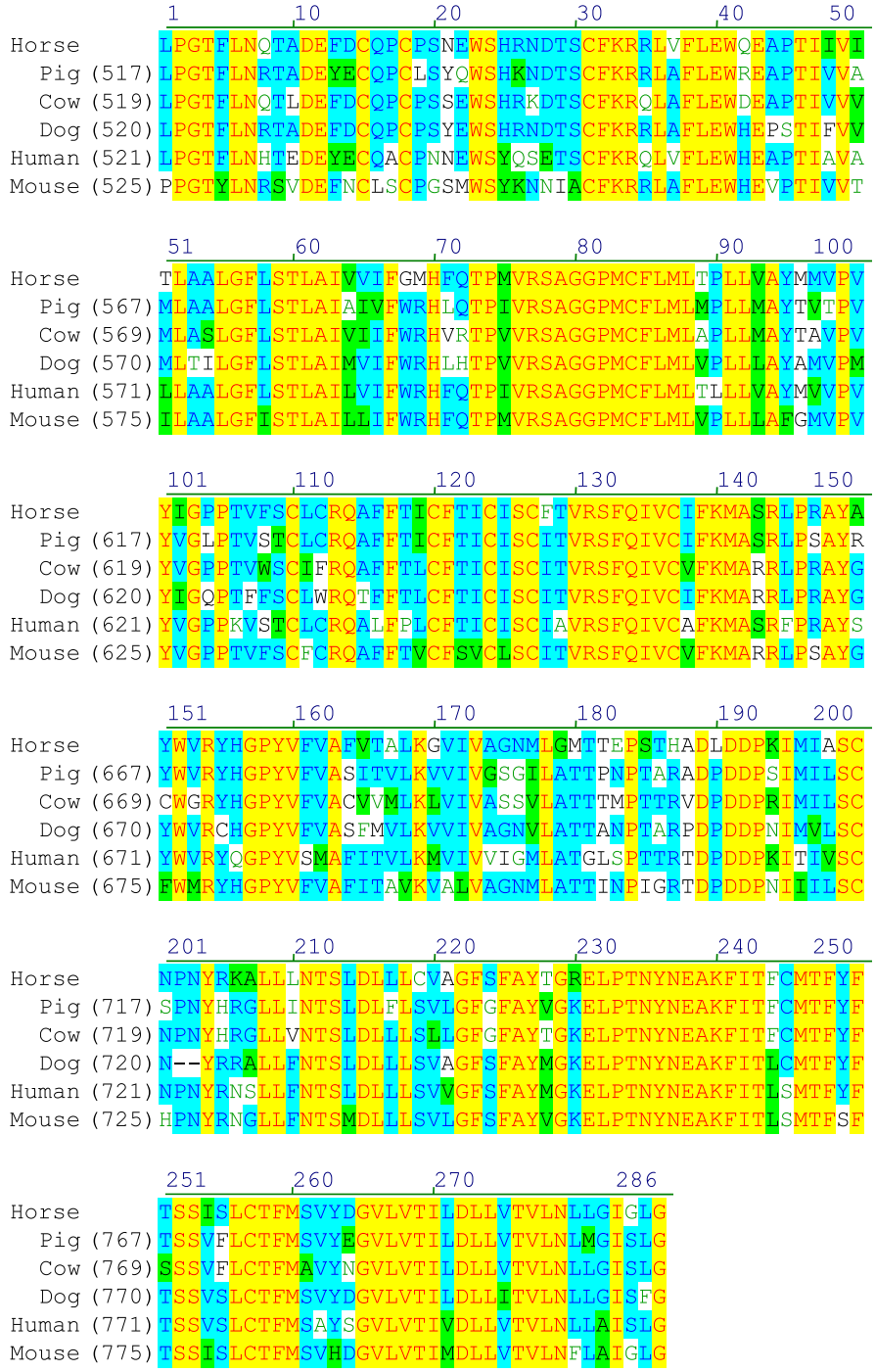

Fig. 1. Alignment of equine T1R2 amino acid sequence (286 residues deduced from cloned mRNA nucleotide sequence) with the corresponding region of pig, cow, dog, human, and mouse T1R2 (numbers in parentheses relate to initiating methionine residue).

in Fig. 5, SGLT1 is expressed on the luminal membrane of all the villus enterocytes, while T1R2 is present in discrete, individual cells among the epithelial cells of the same tissue.

GLP-1 and GLP-2 Are Secreted From Mouse Small Intestine in Response to Glucose and the Artificial Sweetener Sucralose

For experimental convenience, mouse intestine was used as an ex vivo model to determine the ability of the intestine to secrete GLP-1 and GLP-2 in response to glucose or sucralose. Sections of intestinal tissue, with serosa removed, were exposed to either 10% (wt/vol) glucose or varying concentrations of sucralose (2–50 mmol/l). As shown in Fig. 6, GLP-1 and GLP-2 secretion was significantly increased by glucose compared with untreated control tissue; GLP-1 secretion was 23 ± 4 pmol/l ($n = 6$) in response to media glucose compared with 9 ± 1 pmol/l in control ($n = 6$) ($P < 0.01$) (Fig. 6*A*, solid bars). In response to media glucose, GLP-2 secretion was 322 ± 40 pmol/l ($n = 6$) vs. 175 ± 15 pmol/l in control ($n = 6$) ($P < 0.01$) (Fig. 6*B*, solid bars). Sucralose enhanced GLP-1 and GLP-2 release in a dose-dependent manner at 10, 20, and 50 mmol/l, with maximal effect observed at 50 mmol/l. GLP-1 release increased to 32 ± 5 pmol/l ($n = 6$) compared with 10 ±

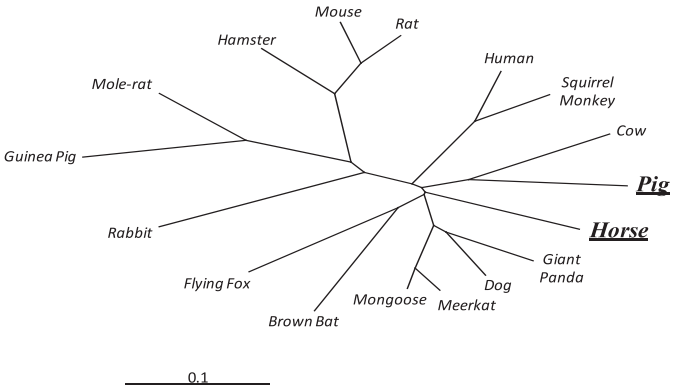

Fig. 2. Radial phylogram, derived from amino acid sequences, depicting the phylogenetic relationships of equine and porcine T1R2 to various mammalian homologs. The scale bar represents the number of substitutions per amino acid position.

1 pmol/l in control ($n = 6$) ($P < 0.001$) (Fig. 6*C*, solid bars), while GLP-2 secretion was 446 ± 44 pmol/l ($n = 8$) vs. 202 ± 19 pmol/l in control ($n = 8$) ($P < 0.001$) (Fig. 6*D*, solid bars). To determine the effect of gurmarin on GLP-1 and GLP-2 release in response to glucose and sucralose, sections of mouse small intestine were preincubated with 5-μg/ml gurmarin for 30 min before addition of 10% (wt/vol) glucose or 20 and 50 mmol/l sucralose. In all samples, preincubation of tissue with gurmarin significantly inhibited GLP-1 and GLP-2 release. GLP-1 and GLP-2 release in response to glucose decreased from 23 ± 4 pmol/l ($n = 6$) to 10 ± 3 pmol/l ($n = 6$) ($P < 0.05$) and from 322 ± 40 pmol/l ($n = 8$) to 195 ± 30 pmol/l ($n = 8$) ($P < 0.05$), respectively (Fig. 6, *A* and *B*, open bars). For tissues exposed to 20 and 50 mmol/l sucralose, preincubation with gurmarin decreased GLP-1 secretion from 19 ± 3 pmol/l ($n = 6$) to 10 ± 3 pmol/l ($n = 6$) ($P < 0.05$) and from 32 ± 5 pmol/l to 12 ± 2 pmol/l ($n = 6$) ($P < 0.001$), respectively (Fig. 6*C*, open bars). In tissues exposed to 20 and 50 mmol/l sucralose, preincubation with gurmarin reduced GLP-2 release from 383 ± 34 pmol/l ($n = 8$) to 271 ± 51 pmol/l ($n = 8$) ($P < 0.05$), and from 446 ± 44 pmol/l ($n = 8$) to 296 ± 61 pmol/l ($n = 8$) ($P < 0.01$), respectively (Fig. 6*D*, open bars). No effect on GLP-1 or GLP-2 secretion from mouse intestinal tissue was observed with gurmarin alone.

DISCUSSION

It was previously demonstrated that the gut epithelium can sense luminal sugars to modulate glucose absorptive capacity, by regulating the expression of the intestinal glucose cotransporter 1, SGLT1 (16, 41). However, until recently, the nature of the intestinal glucose sensor and the downstream signaling pathways were unknown. Subsequently, it was shown that T1R2-T1R3 and α-gustducin, components of the sweet taste receptor in taste buds of the lingual epithelium (33), are coexpressed in endocrine cells of the intestine in a range of species (3, 13, 15, 30, 32). These cells also express GLP-1, GLP-2, and GIP, carbohydrate-responsive gut hormones secreted by L- and K-enteroendocrine cells, respectively (30, 32). Collectively, these results suggested that certain populations of enteroendocrine cells possess a receptor that is capable of sensing the monosaccharide composition of the luminal content and that the T1R2-T1R3 receptor is the most likely candidate for the role of intestinal sweet sensor.

Convincing evidence for the involvement of the sweet taste receptor and gustducin in intestinal sweet transduction was provided by studies using mice in which the genes for either T1R3 or α-gustducin were deleted. Eliminating sweet

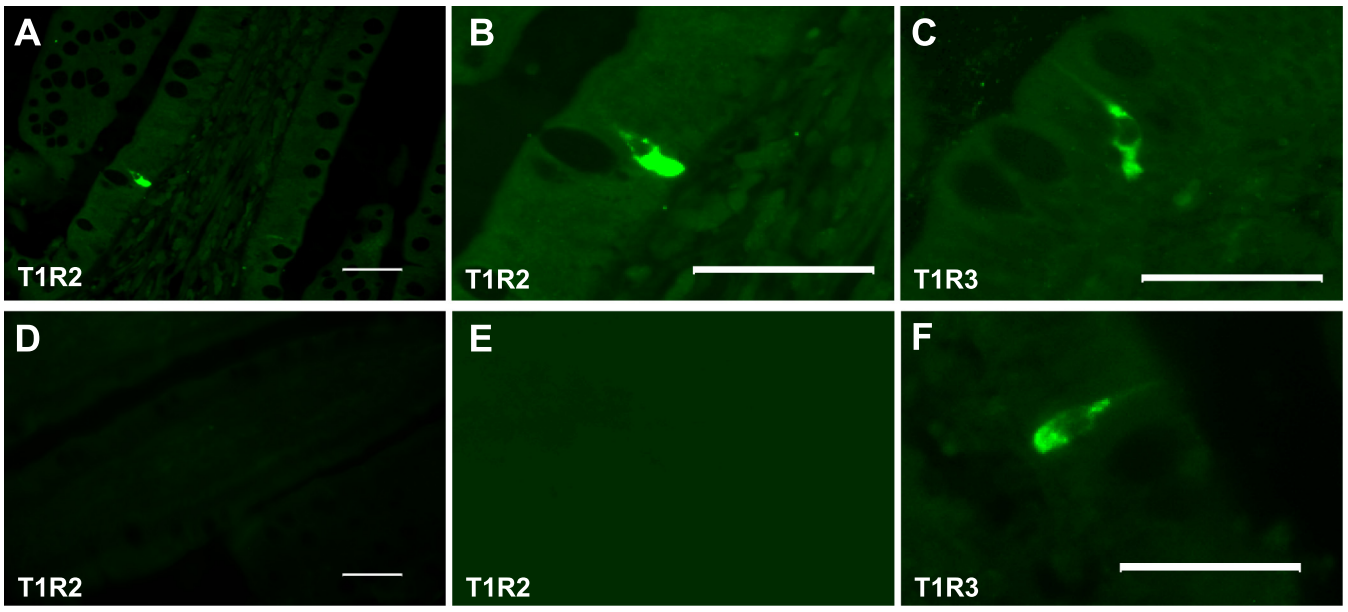

Fig. 3. Representative images showing immunofluorescent detection of T1R2 and T1R3 in horse (*A–C*) and cat (*D–F*) midintestine. Serial sections of horse and cat mid-small intestine, prepared in an identical manner, were probed with antibodies to T1R2 and T1R3. *A* and *B* show T1R2 expression in a discrete, individual cell in the horse intestine. In contrast, *D* and *E* demonstrate that the same T1R2 primary antibody does not bind to any proteins in cat intestine. *C* and *F* demonstrate that T1R3 is expressed in the intestinal tissue of both horse and cat. ×400 magnification (*A* and *D*); ×1,000 magnification (*B*, *C*, *E*, and *F*); scale bar = 10 μm.

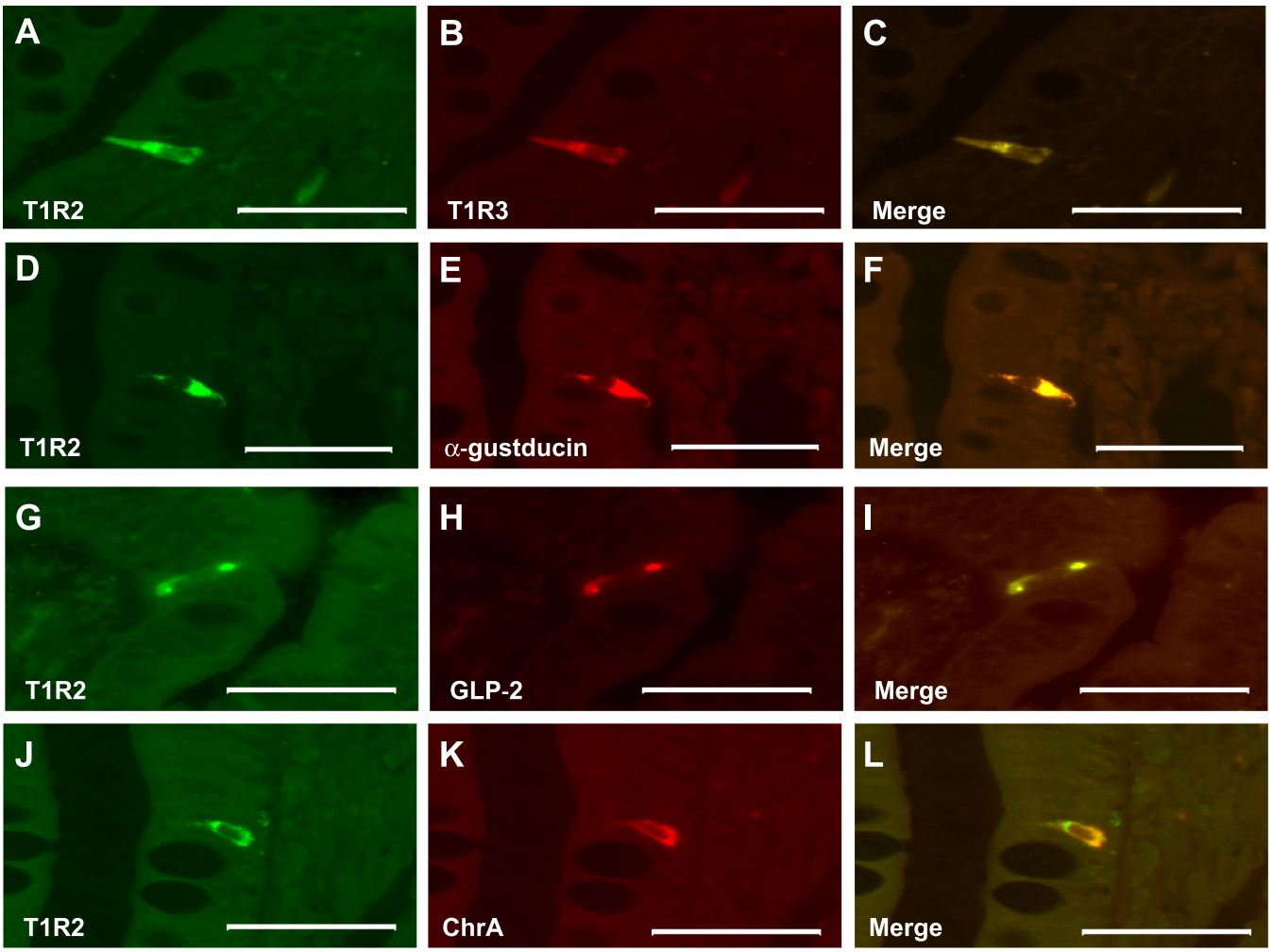

Fig. 4. A typical figure showing colocalization of equine T1R2 (green; *A*, *D*, *G*, and *J*) with T1R3 (red; *B*), α-gustducin (red; *E*), GLP-2 (red; *H*) and ChrA (red; *K*) in horse small intestine, as determined by double-immunofluorescent detection. Images have been merged and show colocalization in the same cells (yellow; *C*, *F*, *I*, and *L*). ×1,000 magnification; scale bar = 10 μm. A similar colocalization pattern for equine T1R3 with T1R2, α-gustducin, GLP-2, and ChrA was also observed.

taste transduction in mice, in vivo, prevented the dietary monosaccharide-induced upregulation of SGLT1 expression that was observed in wild-type mice (13, 30). Furthermore, artificial sweeteners, when included in the diet, also enhanced SGLT1 expression in wild-type mice (30). Supplementation of diets fed to piglets with artificial sweeteners also enhances SGLT1 expression and capacity of the porcine gut to absorb glucose (32), indicating that the artificial sweeteners' effect extends to other species. Natural sugars and artificial sweeteners directly activate gut-expressed T1R2-T1R3, since infusion of the intestine with either glucose or saccharin results in SGLT1 upregulation (42, 45).

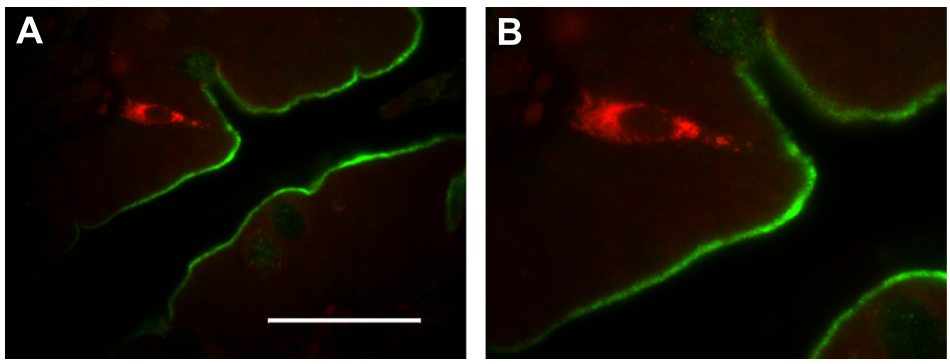

Fig. 5. Expression of SGLT1 and T1R2 in equine intestine. *A*: representative figure showing SGLT1 (green), expressed on the luminal membrane of enterocytes along the entire villus and T1R2 (red), expressed in a discrete, individual cell in horse small intestine, as determined by double-immunofluorescent detection. ×1,000 magnification; scale bar = 10 μm. *B*: enlarged area of the section in *A* showing T1R2 (red) expressed in a typically shaped enteroendocrine cell.

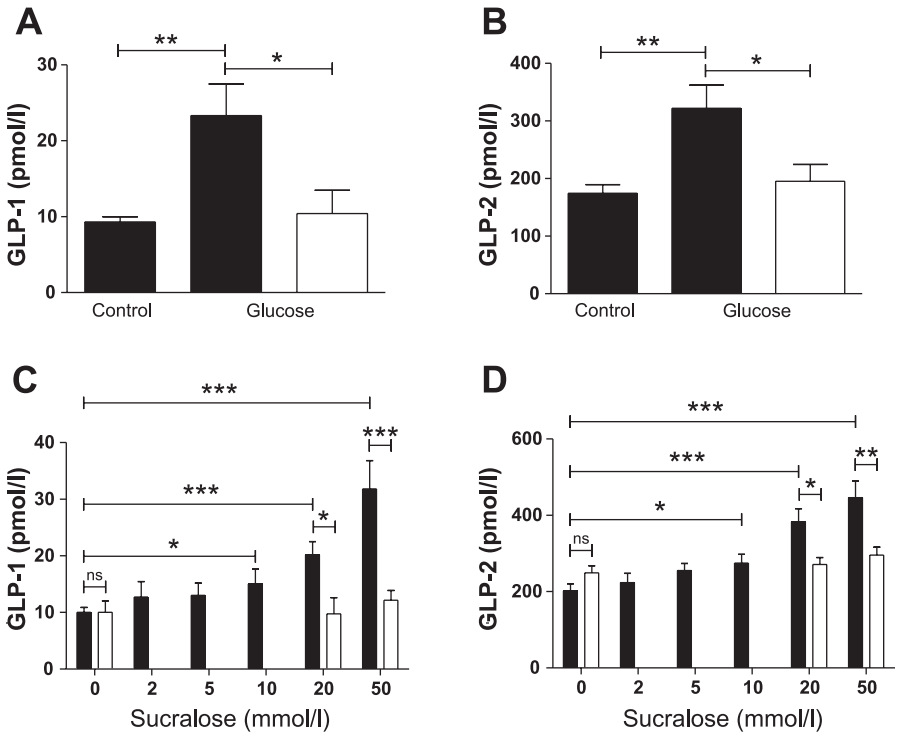

Fig. 6. GLP-1 and GLP-2 secretion from mouse small intestine in response to glucose and sucralose. *A* and *B*: mouse small intestinal tissues (2 cm) were incubated for 1 h at 37°C in incubation media supplemented with 10% (wt/vol) glucose or were untreated (control), in the absence (solid bars) or presence (open bars) of 5 μg/ml gurmarin. *C* and *D*: mouse intestinal tissues (2 cm) were incubated for 1 h at 37°C in incubation media supplemented with the indicated concentrations of sucralose or were left untreated (control), in the absence (solid bars) or presence (open bars) of 5 μg/ml gurmarin. Data are presented as means ± SE. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; ns not significant.

The *Tas1R2* gene is an unexpressed pseudogene in cats (27), and cats do not express T1R2 protein in their lingual or intestinal epithelia (3). It has also been shown that cats are unable to enhance intestinal SGLT1 expression in response to increased dietary carbohydrate (6). As both subunits of heteromeric T1R2-T1R3 are required for sweet responsiveness, the loss of *Tas1R2* in cats provides a genetic explanation for the lack of both sweet preference (27) and response of SGLT1 to changes in dietary carbohydrates (6). Thus, in this "naturally occurring T1R2 knockout" model, there is a good correlation between the absence of T1R2 and the inability to increase SGLT1 expression in response to increased dietary sugars.

Glucose absorbed in the small intestine provides an important source of energy for the horse in intense work (1). We have shown that transport of glucose in equine small intestine is entirely accomplished by SGLT1 (12); this also activates salt and water absorption (22). We have demonstrated that expression of equine SGLT1 is upregulated in response to increased dietary carbohydrates (12). Thus, enhancing SGLT1 expression results in increased glucose and water absorption. In the light of these findings, we were interested in identifying whether the equine intestine expresses the sweet-sensing components shown to be required for SGLT1 upregulation in other species.

The genes for equine T1R3 and α-gustducin have been identified from the horse genome sequence to be located on chromosomes 2 and 4, respectively (EquCab2.0; released 8/31/2011). Phylograms depicting the relationships of the predicted equine amino acid sequences for T1R3 and α-gustducin with homologous sequences from other species (mammals, reptiles, fish, and birds) are shown in Fig. 7. For both genes, the horse amino acid sequences can be seen to cluster within the mammalian clades.

The gene for T1R2, however, has not been identified from the horse genome sequence. In most genome sequences currently available, the *Tas1R2* gene resides between two adjacent genes, *ALDH4A1* and *PAX7*. It has been reported that, in the horse, the *ALDH4A1* and *PAX7* genes are positioned next to each other on chromosome 2, leading to the suggestion that the *Tas1R2* gene has been lost from the horse genome (50). However, as shown here, we have successfully cloned a fragment of T1R2 mRNA from equine lingual epithelium. The amino acid sequence deduced from the nucleotide sequence shows significant homology and phylogenetic similarity to T1R2 amino acid sequences from other mammalian species (Figs. 1 and 2); the closest related homologs being T1R2 of the pig and cow (>85% similarity, >75% identity) (Fig. 2). Furthermore, using a characterized antibody to T1R2, we show by immunohistochemistry that T1R2 protein is expressed in enteroendocrine cells of the equine small intestine, and is coexpressed with both T1R3 and α-gustducin (Figs. 3 and 4). Moreover, double immunofluorescent labeling shows that SGLT1 and T1R2 are both expressed in equine small intestine (Fig. 5), with SGLT1 residing on the luminal membrane of enterocytes along the entire villus and T1R2 in a subset of cells, as shown in other species (30, 32).

Our investigations into the genomic location of the horse *Tas1R2* gene indicate that there is a region of ~98 kb between *ALDH4A1* and *PAX7* on equine chromosome 2 (NCBI accession number NC_009145). Within this region, there is an area of unknown sequence, over 29 kb in length, some 3 kb from the 3′-end of the *ALDH4A1* gene. Analysis of the *Tas1R2* gene and its location relative to the *ALDH4A1* gene in other mammalian species (cow, dog, human, and mouse) shows that the *Tas1R2* gene varies in size from 12–20 kb and is located 3–12 kb from the 3′-end of the *ALDH4A1* gene. With this information in

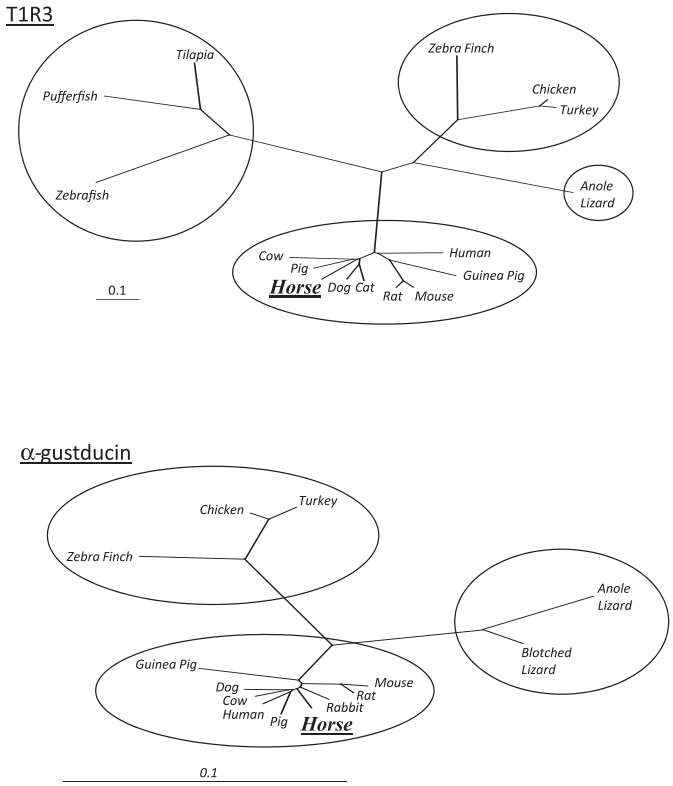

Fig. 7. Radial phylograms, derived from amino acid sequences, depicting the phylogenetic relationships of equine T1R3 and α-gustducin to homologs from various species (mammals, reptiles, fish and birds). The scale bars represent the number of substitutions per amino acid position.

mind, combined with the positive identification of equine T1R2 mRNA and protein presented here, we conclude that the equine *Tas1R2* gene is present in the horse genome and its most likely location is on chromosome 2, between *ALDH4A1* and *PAX7*, within the 29 kb region of unknown sequence.

In support of our findings, it has been reported, using a two-bottle preference test, that horses show a preference for sucrose, demonstrating the ability of the horse to taste sugar (35). Moreover, we have shown that expression of SGLT1 is upregulated in the intestine of horses maintained on a grain (starch) diet, compared with those fed a grass diet, indicating that horses have the ability to enhance their intestinal capacity to absorb glucose in response to increased dietary carbohydrates (12).

In this paper, we show that cells that express T1R2-T1R3 and α-gustducin also possess the carbohydrate-responsive gut hormone GLP-2 (Fig. 4). These cells also possess chromogranin A (Fig. 4), a classical marker of enteroendocrine cells (17), indicating that the sweet receptor elements and GLP-2 are expressed in equine enteroendocrine cells, as shown in other species (30, 32). There is a large body of evidence indicating that intravenous/systemic infusion of GLP-2 results in SGLT1 upregulation (8, 9, 34, 39). Here, we show that GLP-2 (and GLP-1) is secreted through sugar/sweetener activation of the T1R2-T1R3 receptor. Exposure of mouse intestinal tissue to either glucose or the artificial sweetener, sucralose, resulted in secretion of GLP-1 and GLP-2. Moreover, this secretion was inhibited in the presence of gurmarin, a specific inhibitor of the sweet receptor that binds to the venus fly trap domain of T1R3 and inhibits its function (29, 30, 40) (Fig. 6). Furthermore, the levels of GLP-1 and GLP-2 released by control and glucose-stimulated tissues are similar to in vivo studies using rats and humans given glucose or maintained as controls (5, 44).

Horses have high levels of intestinal disaccharidases, an ability evolved to digest disaccharides present in the horse's natural grass diet (25). In contrast they have low levels of pancreatic amylase (26, 36). It has been shown that diets containing high concentrations of starch given abruptly to horses can induce intestinal dysfunction. This is due to excess unabsorbed starch entering the large intestine, disrupting the large intestinal microenvironment, thus disposing the horse to health-threatening conditions, such as colic (10, 20, 21, 46). Moreover, high concentrations of glucose given to performing horses may result in a rapid insulin peak, leading to hypoglycemia-induced exhaustion (47, 48). Artificial sweeteners are potent activators of T1R2-T1R3 (28); many are also nonmetabolizable and nonabsorbable. As recently demonstrated (32), artificial sweeteners provide effective dietary supplements for enhancing intestinal glucose absorptive capacity.

The understanding of the functional properties of equine gastrointestinal carbohydrate sensing, digestion, and absorption allows the rational formulation of feed and feed supplements to enhance intestinal glucose and water absorption. This has attendant promise for enhancing equine performance and alleviating postexercise dehydration.

Perspectives and Significance

Hard-working horses have a greater demand for glucose as a source of energy. Enhancing the activity of the intestinal glucose cotransporter 1, SGLT1, not only provides the horse with more glucose, but also salt and water. Equine intestine expresses the sweet-sensing components required for upregulation of SGLT1 in response to increased luminal glucose. The sweet sensor may provide a convenient and accessible target for manipulating the capacity of the equine intestine to absorb glucose (and water). This has potential for enhancing energy uptake and hydration for hard-working horses.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: K.D. and S.P.S.-B. conception and design of research; K.D., M.A.-R., D.K.A., and A.W.M. performed experiments; K.D., M.A.-R., D.K.A., and S.P.S.-B. analyzed data; K.D., M.A.-R., C.J.P., and S.P.S.-B. interpreted results of experiments; K.D. and M.A.-R. prepared figures; K.D. and S.P.S.-B. drafted manuscript; K.D., Y.N., and S.P.S.-B. approved final version of manuscript; C.J.P. and S.P.S.-B. edited and revised manuscript.

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