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Regional and cellular distribution of monocarboxylate transporters 13 and 14 in the cattle gastrointestinal tract

D. Kirat[1] and T. Miyasho[2]

[1] Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.
[2] Department of Veterinary Biochemistry, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan.

Corresponding author: Doaa Kirat, Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt. E-mail: doaakirat@hotmail.com

Abstract

Understanding of the basic function of orphan transporters can only be achieved by examining their cellular location. This study is the first to describe the precise cellular localization of the orphan monocarboxylate transporters (MCT13) and (MCT14) in the physiologically distinct regions of the gastrointestinal tract of mammals. The present study demonstrated conclusively the regional distribution and relative expression levels of MCT13 and MCT14 on both mRNA and protein levels in the cattle gastrointestinal tract. The mRNA expression levels of MCT13 and MCT14 in the rumen, abomasum, jejunum, cecum, and proximal colon of cattle were examined using quantitative real time-PCR analysis. The precise cellular location of MCT13 and MCT14 along each part of the cattle stomach and intestine was carried out by immunohistochemistry. The data reveal distinct regional distribution in gene expression profiles of both MCT13 and MCT14 along the cattle gastrointestinal tract. Our study might be beneficial in future research to understand their physiological role in the ruminant gastrointestinal tract.

Key words: Orphan transprters, Ruminants, Localization, MCT13, MCT14.

Introduction

The gastrointestinal tract has multiple functions including digestion, nutrient absorption, and secretion of hormones. Several studies have evaluated the expression of genes in the ruminant gastrointestinal tract to gain insight into mechanisms involved in nutrient uptake, transport across the epithelial cell membranes, and pH hemostasis along the gastrointestinal tract.

In mammales, fourteen members of the monocarboxylate transporter (MCT, SLC16) gene family have been identified (1). To date, six of the 14 members have been functionally characterized with only MCT1–4 showing proton-coupled monocarboxylate transport, whereas MCT8 and MCT 10 catalyze the sodium and proton-independent transport of thyroid hormone and aromatic acids, respectively (1). The MCT family also includes a eight 'orphan' transporters, the properties and function of which have remained elusive until recently (1).

In ruminants, our previous RT-PCR studies have shown that many MCT family isoforms; MCT1, MCT2, MCT3, MCT4, MCT7, MCT8, MCT9, MCT10, MCT13, and MCT14 are constitutively expressed in the various parts of the gastrointestinal tract (2). Moreover, we demonstrated in detail the exact cellular localization of MCT1, MCT2, MCT4, MCT7, and MCT8 along the ruminant gastrointestinal epithelial cells (2, 3, 4) and proved that MCT1 and MCT4 are important transporters for short-chain fatty acid in the cattle gastrointestinal tract (3, 4).

For the moment however, monocarboxylate transporters MCT13 and MCT14 are orphan members of the MCT family whose substrates and consequently their functions remain unidentified. As a first step in elucidating their functions in mammales, it is critical to ascertain where in the body these transporters are expressed. Determining their regional expression patterns along the gastrointestinal tract will further characterize their transport functions in the gut. Recently, RT-PCR study published by Kirat et al (2) showed gene transcripts and ubiquitous expression for MCT13 and MCT14 in all regions of the cattle gastrointestinal tract, suggesting that both MCT13 and MCT14 might play essential physiological roles in ruminants. Therefore, the aim of the present study was to elucidate the precise cellular localization of MCT13 and MCT14 proteins along the cattle gastrointestinal tract by immunohistochemichal analysis as well as to measure their mRNA expression levels by quantitative real-time PCR.

Materials and methods

Animals

Six adult cattle were used in all experiments. The experimental protocol used in the present study was approved by the Ethics Committee for Animal Experiments in the School of Veterinary Medicine, Rakuno Gakuen University. This committee was established under the Laboratory Animal Control Guidelines, which are basically consistent with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health in the USA (NIH publication no. 86–23, revised in 1985).

Tissue samples

Immediately after killing the animals by bleeding from the carotid artery following intravenous injection with sodium pentobarbital (35 mg kg^{-1}), the gastrointestinal tract was removed from the abdominal cavity.

Samples were collected from all of the gastrointestinal segments; including the rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, cecum, proximal colon, and distal colon. The tissues were washed in ice cold 0.9% (w/v) NaCl (pH 7.0). Ruminal and reticular epithelia were peeled from underlying and connective tissues, while individual omasal plies were removed. In the case of the abomasum and intestinal sections, the epithelium of each region was scraped off using glass slides on ice. All the collected samples were immediately frozen in liquid nitrogen, and subsequently stored at -80°C until use for real time-PCR analysis. For immunohistochemical studies, tissue samples were immediately fixed in 4% paraformaldehyde for 24 h. After fixation, the tissues were dehydrated through a series of graded concentrations of ethanol and xylene, embedded in paraffin, sectioned serially at 4 μm, and mounted on poly-L-lysine-coated slides.

Immunohistochemical analysis

The immunohistochemical localization of MCT13 and MCT14 proteins was performed using Vectastain Elite ABC Kit according to the manufacturer's protocols. After deparafinization, sections were subjected to antigen retrieval by heating for 15 min in a microwave oven in the presence of sodium citrate buffer (0.01 m, pH 6.0). Sections were incubated in 3% (v/v) H_2O_2–methanol at room temperature for 10 min to quench endogenous peroxidase activity, and then washed (3 × 5 min) in PBS, followed by incubation with Block Ace (Dainippon Pharmaceutical Co., Osaka, Japan) at 37°C for 30 min, to prevent non-specific reactions. Subsequently, sections were incubated overnight with the diluted (1 : 10 in PBS) rabbit anti-human MCT13 antibody (AP11410b; immunogen: 405-426 amino acids from the C-terminus; Abgent, San Diego, CA) or the diluted (1 : 50 in PBS) rabbit anti-human MCT14 antibody (HPA040518; immunogen: YTSHEDIGYDFEDGPKDKKTLKPHPNIDGG; Sigma-Aldrich) in a humidified chamber at 4°C. After washing with PBS, sections were further incubated with biotinylated chicken anti-rabbit IgG (GTX26828, GeneTex, San Antonio, Tex., USA) at a dilution of 1: 200 for 30 min. The sections were then washed (3 × 5 min) with PBS, and then treated with 2% avidin–biotin–peroxidase complex (ABC) reagent for 30 min. Afterwards, sections were reacted with 0.5% (w/v) 3,3′-diaminobenzidine tetrachloride (Kanto Chemical Co., Inc., Tokyo, Japan) in PBS containing 0.01% H_2O_2, to visualize the bound antibody, and counterstained with Mayer's haematoxylin.

To verify the localization of MCT13 and MCT14 in the apical and basolateral domains, serial sections were immunostained with antibodies against alkaline phosphatase, as an apical membrane marker (mouse anti-alkaline phosphatase, ab58958; Abcam) and E-cadherin as a basolateral membrane marker (mouse anti-E-Cadherin-2ndGen Predilute antibody, 08-1223; Zymed, San Francisco, CA, USA) using the same technique applied for MCT13 and MCT14. Goat anti-mouse secondary biotinylated antibody (Vector laboratories, Inc.) was used for both markers. Negative immunohistochemical controls were included in each staining run by the use of MCT13 and MCT14 antibodies that had been preabsorbed with 10 μg ml^{-1} of their peptide antigen.

A semi-quantitative estimation of the immunohistochemical staining was carried out as detailed previously (5). This method is based on the estimation of both the staining intensity of each transporter and the abundance of immunoreactive cells among the different epithelial cells of the examined tissues. The number of MCT13 and MCT14-immunopositive cells in the mucosal layer in each section was counted field by field, with at least 20 fields (×25) per specimen. The number of positive cells was subjectively divided into four grades. The different grades included sections containing no positive cells (–), low (+), moderate (++), considerable (+++), high (++++), and abundant (+++++) cells. The staining intensity was graded 0 (absence of staining or staining equivalent to background staining in the negative control), 1 (weak staining), 2 (moderate staining) or 3 (intense staining).

Real-time PCR

Messenger RNA from each region of the ruminant gastrointestinal tract was isolated using MagNA Pure LC mRNA isolation Kit II (Roche Diagnostics GmbH, Germany) and the cDNA was generated by reverse transcription using Transcriptor first strand cDNA synthesis kit (Roche Diagnostics GmbH, Germany) following the manufacturer's instructions.

Real-time PCR was performed on a LightCycler instrument (Roche Diagnostics) using a LightCycler TaqMan master kit with Universal ProbeLibrary probes (Roche, Mannheim, Germany). PCR primers and probes (Roche Universal ProbeLibrary; Roche) were designed using the Probe Finder software from Roche Applied Science available online at the Universal ProbeLibrary Assay Design Center. For bovine MCT13, primer

Table 1. Semi-quantitative assessment of the immunohistochemical results of MCT13 and MCT14 along the cattle gastrointestinal tract.

	MCT13		MCT14	
	No. of positive cells	Intensity of staining	No. of positive cells	Intensity of staining
Rumen	+++++	**3**	+++++	**3**
Abomasum	++++	**3**	+++	**3**
Jejunum	+++	**3**	++++	**3**
Cecum	++	**2**	++	**2**
Prox. Colon	+	**1**	+	**1**

The different grades included sections containing no positive cells (–), few (+), moderate (++), considerable (+++), high (++++), and abundant (+++++) cells. The staining intensity was graded 0 (absence of staining or staining equivalent to background staining in the negative control), 1 (weak staining), 2 (moderate staining) or 3 (intense staining).

pair (sense: 5′- gggggtatcctgactgcac -3′; antisense: 5′-ggtgggttaaggaggtagca -3′) and probe no. 89 (cat. no. 04689143001) were used. For bovine MCT14, primer pair (sense: 5′- atcggagggctcctgaac -3′; antisense: 5′-atacacgttcacggcgtagg -3′) and probe no. 23 (cat. no. 04686977001) were used. β-actin was used for sample validation and normalization of MCT13 and MCT14 expression. For β-actin, primer pair (sense: 5′-ctaaggccaaccgtgaaaag -3′; antisense: 5′-tacatggctggggtgttga -3′) and probe no. 115 were used.

Statistical analyses

Data are expressed as means ± SE. The values were compared with one-way analysis of variance (ANOVA) for multiple comparisons, while the Student's t test was applied when two groups were compared. A value of $P < 0.05$ or less was considered as statistically significant. All data analysis was performed using the VassarStats web site for statistical computation (http://faculty.vassar.edu/lowry/VassarStats.html).

Results

Localization of monocarboxylate transporter 13 and monocarboxylate transporter 14 along the entire length of the ruminant gastrointestinal tract

Using the immunohistochemical technique, both MCT13 and MCT14 proteins expression and localization were precisely observed in all segments of the ruminant gastrointestinal tract including; rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, cecum, and colon. The distribution pattern and cellular localization for MCT13 and MCT14 proteins in the gastrointestinal tissues are the same amongst the different ruminant animals used in this study. Therefore, representative images for cattle gastrointestinal tract are shown in all figures. The immunohistochemical staining for MCT13 and MCT14 revealed a similar cellular pattern of expression along the ruminant gastrointestinal tract as displayed in figures (1-8).

To prove that the antibodies (anti-human MCT13 and MCT14) used in this study can react with cattle, sheep, and goat tissues, we made homology searches using GENETYX-MAC Software, ver.12 (GENETYX Corporation, Tokyo, Japan) and Basic Local Alignment Search Tool, BLAST (http: //blast.ncbi.nlm.nih.gov/Blast. cgi) between the amino acid sequences of the immunogen of each of the MCT antibodies used against the analogous region of the same MCT isoforms in cattle, sheep, and goat. The homology search data verified that MCT13 and MCT14 immunogen sequences are 91% and 95%, respectively conserved in these animals.

Additionally, the specificities of the immunohistochemical reactions were confirmed by the devoid of labeling in the negative control sections after incubation of the slides with either the preabsorbed MCT13 and MCT14 antibodies with the corresponding peptides antigens or incubation without the primary antibody as well as by the fact that the staining results perfectly coincided with the mRNA distribution previously shown by RT-PCR analysis in the cattle gastrointestinal tract (Kirat et al. 2013).

Moreover, to verify the apical and/or basolateral localization of MCT13 and MCT14 in the examined epithelial cells, antibodies to apical and basolateral membrane markers were immunohistochemically used. The results displayed that the apical marker, alkaline phosphatase and the basolateral marker, E-cadherin were specifically localized to their respective plasma membrane domains and that the localization of MCT13 and MCT14 has been verified in the gastrointestinal tract of cattle based on these selective markers. No specific staining for alkaline phosphatase and E-cadherin was seen in the negative control slides.

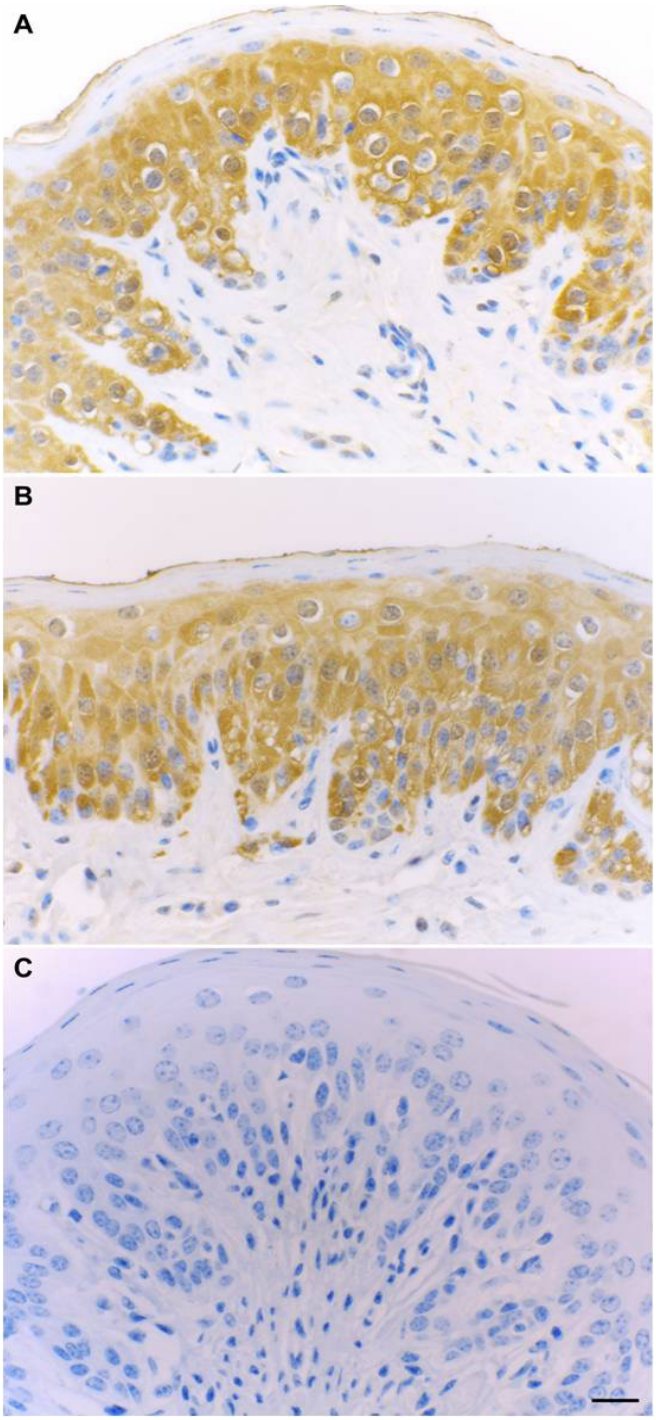

Figure 1. Cellular localization of MCT13 in the cattle forestomach. Immunohistochemical images showed signals for MCT13 in the forestomach (represented by rumen; A, B). Note the localization of MCT13 in the stratum basale and stratum spinosum of the rumen (A, B). (C) is a negative control. Scale bar= 20µm.

Immunohistochemically, the tissue distribution and expression of MCT13 and MCT14 in the various examined tissues were evaluated semi-quantitatively as shown in table (1).

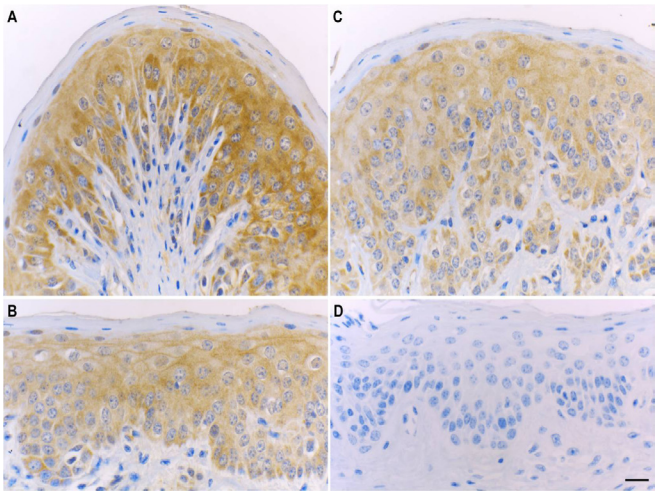

Figure 2. Cellular localization of MCT14 in the cattle forestomach. Immunohistochemical images showed signals for MCT14 in the forestomach (represented by rumen; A-C). Note the localization of MCT14 in the stratum basale and stratum spinosum of the rumen (A-C). (D) is a negative control. Scale bar = 20µm.

Localization of MCT13 and MCT14 in cattle forestomach

In the forestomach, immunohistochemical labeling for MCT13 and MCT14 (Figs. 1 and 2) has been shown to be localized on the strata basale and spinosum of the stratified squamous epithelia of the rumen, reticulum, and omasum. The reticulo-rumen was intensely immunoreactive for MCT13 and MCT14 more than that in omasum.

Localization of MCT13 and MCT14 in cattle abomasum

In the abomasum mucosa, strong MCT13 and MCT14 positivities were found at the surface epithelia and the gastric pits. The immunoreactivities were expressed clearly at the apical and lateral surfaces of the epithelial cells (Figs. 3 and 4).

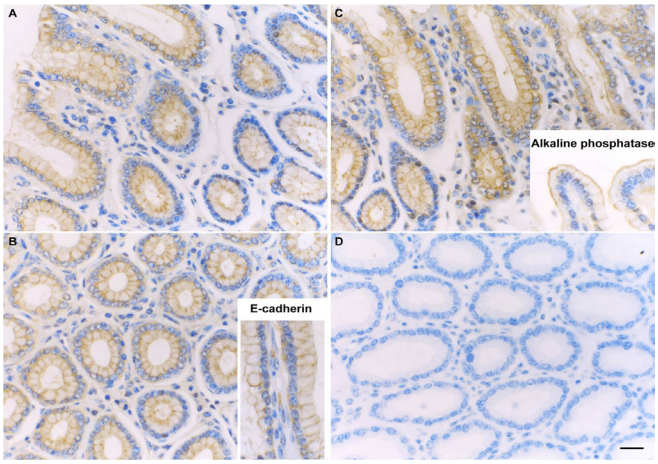

Figure 3. Cellular localization of MCT13 in the cattle abomasum. Immunohistochemical images of MCT13 and selective membrane markers in the cattle abomasum. Serial sections from the abomasum were immunostained with antibodies against MCT13, alkaline phosphatase (an apical membrane marker), and E-cadherin (a basolateral membrane marker). No specific staining for MCT13, alkaline phosphatase, and E-cadherin was seen in the negative control images (D). Scale bars = 20 µm.

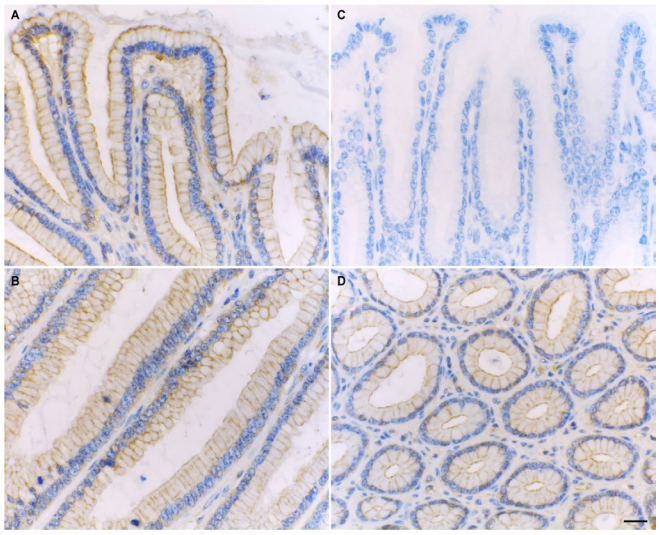

Figure 4. Cellular localization of MCT14 in the cattle abomasum. A, B, and D, immunohistochemical photos showed the localization of MCT14 in the surface epithelia and gastric pit of the cattle abomasum. No specific staining for MCT14 was seen in the negative control images (C). Scale bars = 20 µm.

Localization of MCT13 and MCT14 in cattle small intestine

MCT13 and MCT14-immunopositive and expressing cells in the small intestine were observed along the villus axis and crypts. In all segments of the small intestine (from duodenum to ileum), MCT13 and MCT14 were predominantly expressed on the brush border membranes of the enterocytes over the entire length of the villi (Figs. 5A, B and 6A, C). However in the crypt, MCT13 and MCT14 were mostly found in the apical cytoplasm of the crypt cells in all segments of the small intestine (Figs. 5C and 6B). The immunostaining intensities of both transporters in the small intestine are higher in the villi than that in the crypt. The number of MCT13 and MCT14-immunopositive cells and staining intensity varies with region of small intestine. MCT13

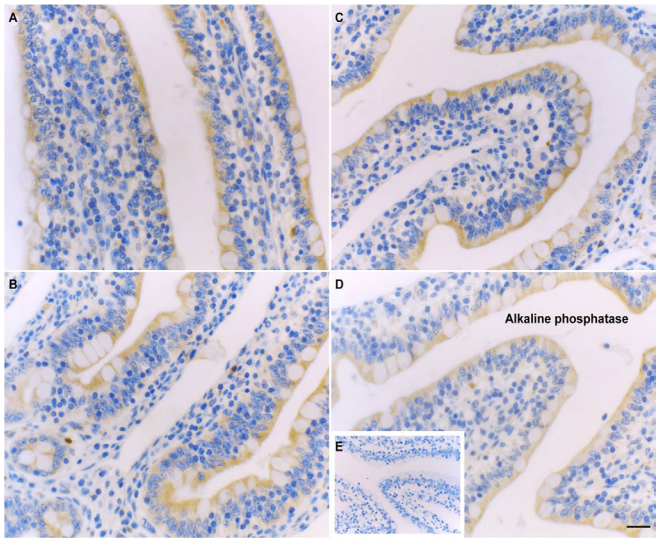

Figure 5. Cellular localization of MCT13 in the cattle small intestine. Immunohistochemical images for serial sections from the small intestine (represented by jejunum) immunostained with antibodies against MCT13 (A-C) and the apical membrane marker; alkaline phosphatase (D). Note the localization of MCT13 on the brush border membranes of the enterocytes over the entire length of the villi (A, B) and in the apical cytoplasm of the crypt cells of the cattle small intestine (C). (E) is a negative control. Scale bar= 20µm.

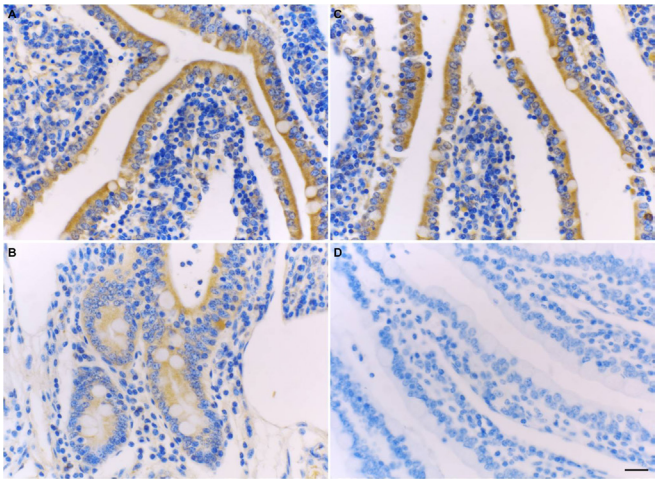

Figure 6. Cellular localization of MCT14 in the cattle small intestine. Immunohistochemical images showed signals for MCT14 in the small intestine (represented by jejunum; A-C). Note the localization of MCT14 on the brush border membranes of the enterocytes over the entire length of the villi (A, C) and in the apical cytoplasm of the crypt cells of the cattle small intestine (B). (D) is a negative control. Scale bar= 20µm.

and MCT14 were most highly expressed in the jejunum (Fig. 5A-C and 6A-C) followed by ileum and to a lesser extent in duodenum (data not shown).

Localization of MCT13 and MCT14 in cattle large intestine

In large intestine (from cecum to colon), MCT13 and MCT14 immunopositive cells were particularly abundant in the surface epithelia, while they were few scattered in the epithelia of crypts (Figs. 7 and 8). The densities of MCT13 and MCT14 were dramatically decreased toward the base of the crypt (Figs. 7 and 8). Both the immunoreactivities of MCT13 and MCT14 signals were localized on the apical and lateral membanes of the surface epithelial cells. While the positive cells in the crypt showed preferentially lateral localization for MCT13 and MCT14 proteins. MCT13 and MCT14 immunopositive cells were intense and abundantly distributed in the cecum (Figs. 7A, B and 8A, B) followed by the proximal colon then distal colon (Figs. 7D, E and 8D, E). In the cecal epithelium, MCT13 and MCT14 are expressed over the cells covering the crypt and the entire surface epithelia (Figs. 7A, B and 8A, B). Whereas, proximal colon and distal colon epithelia localized MCT13 and MCT14 in the surface epithelia and the upper portion of the crypt and absent in those covering the lower portions of crypt (Figs. 7D, E and 8D, E). Additionally, several crypts in the proximal colon and distal colon showed negativity for MCT13 and MCT14 (Figs. 7D, E and 8D, E).

Collectively, the semi-quantitative assessment of the immunohistochemical results (Table 1) showed that MCT13 and MCT14 protein expression were abundant and intense in the all parts of the stomach, jejunum, and cecum and were much less in other portions of the cattle gastrointestinal tract.

The specificities of these immunoreactions on sections were confirmed by a conventional protocol including absorption tests by use of antigens that showed no specific signals for the MCT13 and MCT14 (Figs. 1C,

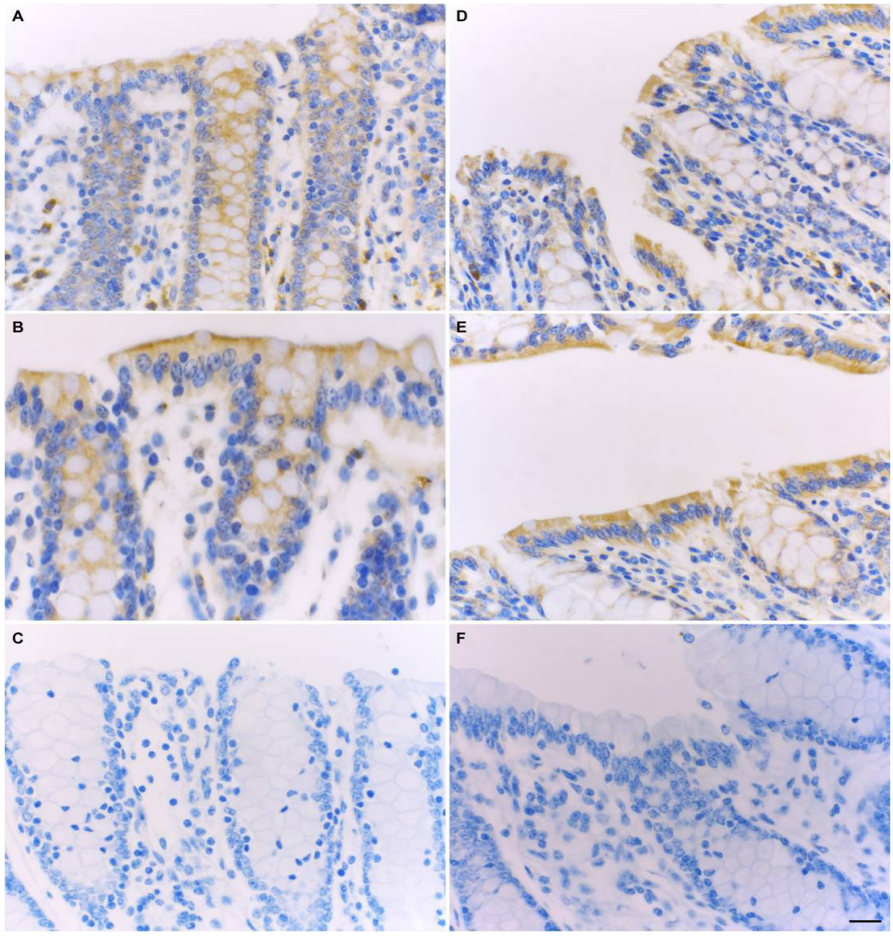

Figure 7. Cellular localization of MCT13 in the cattle large intestine. Immunohistochemical images showed signals for MCT13 in the large intestine (represented by cecum; A, B and proximal colon; D, E). No specific staining for MCT13 was seen in the negative control images (C, F). Scale bars = 20 µm.

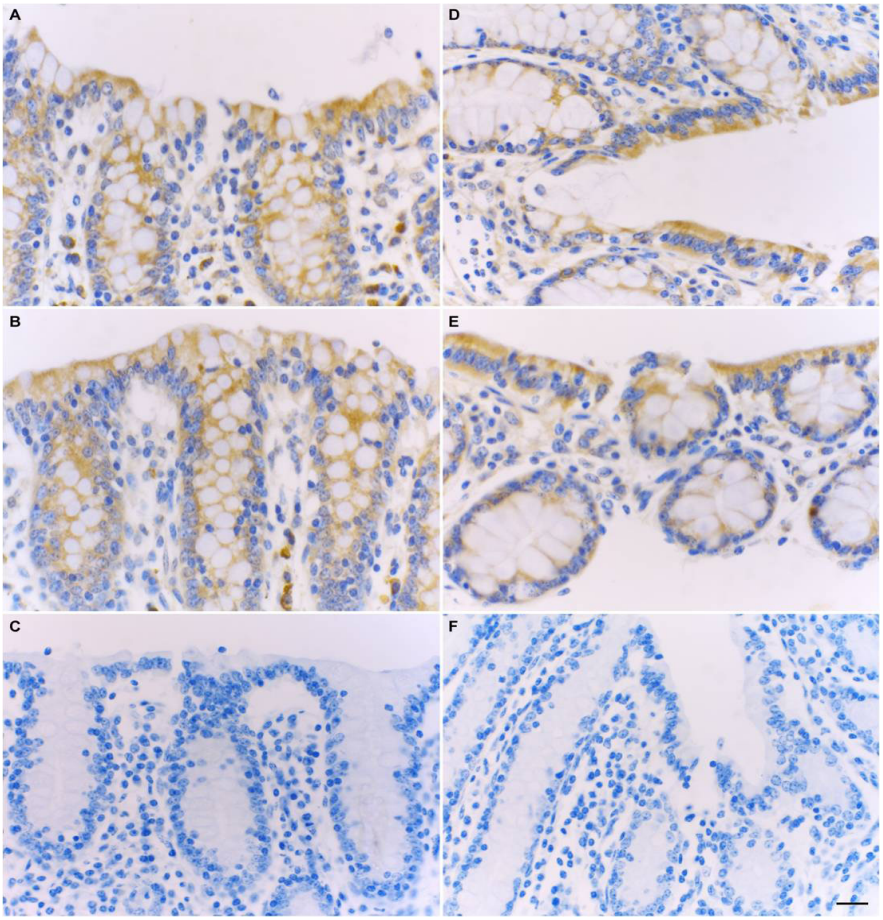

Figure 8. Cellular localization of MCT14 in the cattle large intestine. Immunohistochemical images showed signals for MCT14 in the large intestine (represented by cecum; A, B and proximal colon; D, E). No specific staining for MCT14 was seen in the negative control images (C, F). Scale bars = 20 μm.

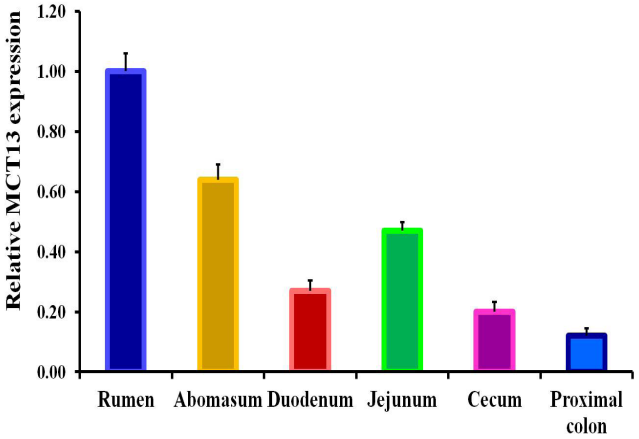

Figure 9. Real-time quantitative PCR analysis of MCT13 expression along the cattle gastrointestinal tract. Bars represent the mean ± S.E. from six animals. * $p \leq 0.05$ vs rumen.

2D, 3D, 4C, 5E, 6D, 7C, F, 8C, F) as well as by the fact that the staining results perfectly coincided with the mRNA distributions previously shown by RT-PCR (Kirat et al. 2013).

Real-Time PCR analysis of monocarboxylate transporter 13 and monocarboxylate transporter 14 along the entire length of the ruminant gastrointestinal tract

To further analyze the tissue expression profile obtained by immunohistochemistry for MCT13 and MCT14 in the ruminant gastrointestinal, real-time PCR was used to compare the levels of each MCT13 and MCT14 mRNA expression in the cattle gastrointestinal regions (Figs, 9 and 10). The results were normalized to a housekeeping gene; β-actin and for the graphic representation, all values for the indicated tissues were compared with rumen (nominally set equal to 1.0). The expression level of the MCT13 mRNA in the cattle GIT was significantly higher in the following order: rumen > abomasum > jejunum > cecum > duodenum > proximal colon (Fig. 9). Whereas MCT14 mRNA transcripts level in the cattle GIT was significantly higher in the following order: rumen > jejunum > abomasum > duodenum > cecum > proximal colon (Fig. 10).

Discussion

Data on where proteins are localized within tissues and cells provide important information as to

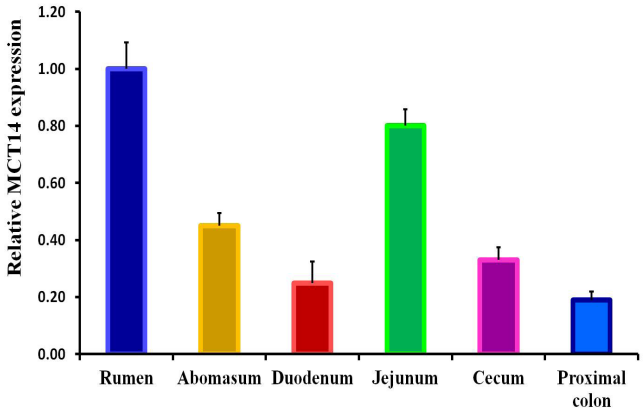

Figure 10. Real-time quantitative PCR analysis of MCT14 expression along the cattle gastrointestinal tract. Bars represent the mean ± S.E. from six animals. * $p \leq 0.05$ vs rumen.

what basic functions a protein may have. The present study demonstrated conclusively, for the first time, the regional distribution and expression levels of MCT13 and MCT14 along the cattle gastrointestinal tract and showed the precise cellular localization of their proteins in all parts of the cattle gastrointestinal tract. Our immunohistochemical analyses demonstrate that MCT13 and MCT14 approximately have the same cellular distribution pattern in the vast majority of the analyzed tissues, implying that these two genes may directed towards the same functional role.

MCT13 and MCT14 are orphan members of the solute carrier family 16 for monocarboxylates, thyroid hormone, and aromatic amino acids. The phylogenetic analysis of the MCT members which based on the amino acid sequence provides valuable information regarding the functional clustering of the MCT family. The predicted phylogeny of MCTs family members reveals that MCT1-MCT4 have a high degree of sequence similarity. Furthermore, the MCT1-MCT4 that mediate the proton-linked transport of metabolically important monocarboxylates are associated in the same cluster. This cluster is sub-divided into two shorter branches, the MCT1-MCT2 and MCT3-MCT4, which correlate with their range of substrate specificity and affinities found for the mammalian transporter isoforms (6). Additionally, MCT8 and MCT10 that mediate the transport of thyroid hormones (7) and aromatic amino acids (8), respectively in a proton and sodium independent manner, share a closer phylogenetic relationship. Therefore, it is possible due to its relatedness to MCT8 and MCT10 in the MCT phylogram, MCT14 may be involved in amino acid transport. Additionally, Halestrap (1) declared that the key residues required for proton translocation are not present in MCT14 and that MCT13 is likely to be proton-linked transporter.

Localization of orphan members of the MCT family in ruminant tissues will have a significant impact on our understanding of cell and organ physiology throughout the body. The present study may provide foundation data for further mechanistic characterization of MCT13 and MCT14 proteins in the future. The possible role(s) and involvement of MCT13 and MCT14 in the physiological and pathological conditions of the gastrointestinal tract remain to be determined. Our study might be beneficial in future research to understand their physiological role in the ruminant gastrointestinal tract.

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