Available online at www.sciencedirect.com
SCIENCE DIRECT®

The Veterinary Journal 171 (2006) 462–467

The Veterinary Journal

www.elsevier.com/locate/tvjl

Monocarboxylate transporter 1 gene expression in the ovine gastrointestinal tract

D. Kirat [a], H. Inoue [a], H. Iwano [b], K. Hirayama [c], H. Yokota [b], H. Taniyama [c], S. Kato [a,*]

[a] *Department of Veterinary Physiology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan*
[b] *Veterinary Biochemistry, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan*
[c] *Veterinary Pathology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan*

Accepted 3 December 2004

Abstract

In this study, we investigated the tissue distribution and expression of monocarboxylate transporter 1 (MCT1) along the gastrointestinal tract of sheep. Western blot analysis suggested the presence of MCT1 as a 43-kDa protein in immunoblots of membranes from the various tissues examined. The results of Western blotting were further confirmed by immunohistochemical studies, which revealed intense immunoreactivity for the MCT1 protein in the forestomach (rumen, reticulum and omasum) and large intestine (caecum, proximal and distal colon). Moderate reactivity, however, was detected in the abomasum, while no immunoreactivity could be seen in any regions of the small intestine examined. Furthermore, MCT1 was expressed at the mRNA level as determined by reverse transcriptase polymerase chain reaction (RT-PCR), which showed a band of the expected size (300 bp) in all tissues examined. From these results we concluded that MCT1 protein is highly expressed and distributed in the stomach and large intestine of sheep suggesting that MCT1 may play a significant role in the transport of short chain fatty acids and their metabolites in the gastrointestinal tract of ruminants.

Keywords: Monocarboxylate transporter 1 (MCT1); Sheep; Gastrointestinal tract; Western blot; Immunohistochemistry; RT-PCR

1. Introduction

Short chain fatty acids (SCFA; acetate, propionate, and butyrate) are the major anions in the ruminant gastrointestinal tract; they are produced by anaerobic microbial fermentation of dietary carbohydrates and provide up to 80% of the energy needs of the animal for maintenance, growth, and lipogenesis (Bergman, 1990). In addition to their function in energy metabolism, SCFA absorption plays a major role in pH regulation (Gäbel and Sehested, 1997).

Cell membranes are relatively impermeable to SCFA anions and a carrier-mediated transport mechanism(s) is needed to transport them across these structures (Rechkemmer et al., 1995). Previous studies have demonstrated the presence of a bicarbonate-dependent, carrier-mediated anion exchange mechanism for SCFA transport in the rat distal colon (Mascolo et al., 1991), human colon (Harig et al., 1996), rumen (Gäbel and Sehested, 1997), and rabbit ileum (Manokas et al., 2000). However, in the past few years, a family of monocarboxylate transporters (MCT) that represents a gene family of fourteen isoforms has been cloned and expressed in a variety of tissues each having different tissue distribution, of which only the first four (MCT1–MCT4) have been

---

[*] Corresponding author. Tel.: +81 11 388 4734; fax: +81 11 387 5890.
E-mail address: kato@rakuno.ac.jp (S. Kato).

1090-0233/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tvjl.2004.12.009

demonstrated experimentally to catalyse the proton-linked transport of lactate, pyruvate and ketone bodies across the plasma membrane (Halestrap and Meredith, 2004; Halestrap and Price, 1999).

Among the MCT isoforms, MCT1 appears to be the archetypical monocarboxylate transporter. Studies on epithelial cells of the gastrointestinal tract of monogastric animals and humans (Garcia et al., 1995; Orsenigo et al., 1999; Price et al., 1998; Ritzhaupt et al., 1998; Takanaga et al., 1995) as well as on the sheep rumen (Müller et al., 2002) suggested that only the MCT1 isoform plays a major role in the transport of various monocarboxylates, whereas other MCT isoforms seem to be of little or no importance.

Characterization of endogenous MCT1 in erythrocytes (Poole and Halestrap, 1993), in addition to MCT1 expression in Xenopus oocytes (Bröer et al., 1998; Manning Fox et al., 2000), has revealed that it transports a number of short chain (C-2 to C-5) monocarboxylates. Studies carried out by Ritzhaupt et al. (1998) using human and pig colon, and also by Hadjiagapiou et al. (2000) using Caco-2 cells, have provided additional evidence that MCT1 may play a role in butyrate transport. Juel and Halestrap (1999) indicated that MCT1 mediates the concerted translocation of $1H^+$ and a monocarboxylate anion by an ordered mechanism in which H^+ binding precedes monocarboxylate binding.

Until recently, little was known about the tissue distribution of MCT1 in ruminants. Therefore, we aimed to examine the expression of MCT1 along the ovine gastrointestinal tract (the four compartments of the stomach, the small intestine and the large intestine) at both the protein level using Western blotting and immunohistochemistry, and at the mRNA level using RT-PCR.

2. Materials and methods

2.1. Animals

Three mature male Suffolk-strain sheep (68–76 kg) were used in this study. The animals were placed in individual pens and fed hay (100 g) and Lucerne alfalfa) pellets (2.5% of body weight) daily until slaughter. Animals were euthanased by bleeding using a cannula inserted into the carotid artery following intravenous injection with sodium pentobarbital (35 mg/kg).

All animals were treated in accordance with the Laboratory Animal Control Guidelines of Rakuno Gakuen University, which conform to the *Guide for the Care and Use of Laboratory Animals of the National Institute of Health* in the USA (NIH publication No. 86-23, revised 1985).

2.2. Tissue preparation

Tissue samples from the four stomach compartments (rumen, reticulum, omasum, and abomasum), small intestine (duodenum, jejunum, and ileum) and large intestine (caecum, proximal colon, and distal colon) were immediately collected from the animals after slaughter. The tissues were washed in ice cold 0.9% (w/v) NaCl (pH 7.0). Ruminal and reticular epithelia were peeled from the 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 free using glass slides on ice. All collected samples were immediately frozen in liquid N_2, and subsequently stored at −80 °C until used for Western blotting and RT-PCR analyses. 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.

2.3. Polyacrylamide gel electrophoresis and western blot analysis

Mucosal samples were homogenized by a physcotron in hypotonic buffer (20 mM Tris–HCl at pH 7.4, 5 mM $MgCl_2$, 1 mM sodium EDTA, 1 mM dithiothreitol) containing a protease inhibitor cocktail (Nacalai Tesque Inc) at a ratio of 5 mL of buffer/g tissue and centrifuged at 200*g* for 10 min at 4 °C. The resulting supernatant was then centrifuged at 200,000*g* for 30 min at 4 °C, and the membrane pellet was resuspended in a buffer containing 62.5 mM Tris–HCl at pH 6.8, 15% (w/v) SDS, 8 M urea, 10% (w/v) sucrose, 100 mM dithiothreitol, and 10 mM sodium EDTA.

Protein concentration was measured (Lowry et al., 1951) following precipitation of samples with 20% (w/v) trichloroacetic acid in 0.015% (w/v) sodium deoxycholate. Aliquots (20 μg) of membrane protein from the indicated tissue were separated on a 10% SDS–polyacrylamide gel (Laemmli, 1970) and transferred to nitrocellulose membranes (Toyo Roshi Kaisha, Ltd). The membranes were blocked overnight at 4 °C with 5% (w/v) nonfat dry milk in PBS-T (0.1% Tween 20 in phosphate-buffered saline) and then probed with the primary antibody (chicken anti-MCT1; AB1286; Chemicon International Inc), at a dilution of 1:1000, for 1 h in PBS. After washes in PBS-T, the membranes were incubated for 30 min with rabbit anti-chicken IgY conjugated to horseradish peroxidase (Upstate) diluted 1:2000 in PBS.

Immunodetection was performed by chemiluminescence (ECL, Amersham International). Negative control studies were carried out by the preabsorption of the