Research Note

Partial Na^+ Dependence of DL-2-Hydroxy-4-(Methylthio)Butanoic Acid Uptake in the Chicken Small Intestine[1]

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ABSTRACT Methionine hydroxy analogue DL-2-hydroxy-(4-methylthio)butanoic acid (HMTBA) is commonly used as a supplemental source of Met in commercial animal diets. To better understand the uptake of this analogue by the chicken intestine, the aim of the present study was to assess the contribution of Na^+ gradient on HMTBA accumulation in everted sacs of the chicken small intestine (duodenum, jejunum, and ileum). In the presence of an H^+ gradient, uptake was lower in the absence of Na^+ along the chicken small intestine, although no significant differences were detected in the duodenum. In contrast, in the absence of an H^+ gradient, no significant differences were detected between the 2 Na^+ conditions. In conclusion, the observed relationship between Na^+ and H^+ dependence indicates the participation of the apical Na^+/H^+ exchanger in HMTBA uptake in the chicken small intestine.

Key words: DL-2-hydroxy-4-(methylthio)butanoic acid, methionine hydroxy analogue, H^+-dependent transport, chicken everted sac, Na^+/H^+ exchanger

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INTRODUCTION

Methionine hydroxy analogue DL-2-hydroxy-(4-methylthio)butanoic acid (**HMTBA**) is a synthetic source of Met that is widely used to supplement commercial animal diets and thus improve the nutritional value of proteins deficient in S amino acids. The HMTBA is transported across the apical membrane of chicken enterocytes by an H^+-dependent carrier-mediated mechanism (Maenz and Engele-Schaan, 1996; Pan et al., 2002; Martín-Venegas et al., 2006b) related to L-lactate transport (Brachet and Puigserver, 1987, 1989). Recently, we have characterized HMTBA uptake across Caco-2 cell apical membrane, and the results indicate the participation of a transport mechanism with many of the properties of the H^+-dependent monocarboxylate transporter 1 (Martín-Venegas et al., 2007).

The Na^+/H^+ exchangers (**NHE**) comprise a family of highly related proteins that mediate the electroneutral 1:1 exchange of intracellular H^+ for extracellular Na^+ across the membrane (Orlowski and Grinstein, 2004). In mammalian intestine, NHE2 and NHE3 are predominantly located in the apical membrane, and both are mainly related with absorptive functions that influence systemic electrolyte homeostasis (Zachos et al., 2005). In contrast, NHE1 is selectively expressed in the basolateral membrane and is believed to have an important role in intracellular pH and volume regulation (Slepkov et al., 2007). In the chicken, both NHE2 and NHE3 have also been reported in the apical membrane, as well as NHE1 in the basolateral domain (Bhartur et al., 1997; De la Horra et al., 1998; Donowitz et al., 1998).

Apical NHE3 plays an important role in the maintenance of an acidic microclimate in the close vicinity of the cell surface, which constitutes the driving force for H^+-dependent transport systems (Gonda et al., 1999; Thwaites et al., 1999; Orlowski and Grinstein, 2004). Characterization of HMTBA transport in Caco-2 cells confirms the cooperation between monocarboxylate transporter 1 and apical NHE3 (Martín-Venegas et al., 2007). Given the widespread use of HMTBA in animal production, it is important to understand the mechanism of its absorption. Taking into account that NHE3 activity is dependent on extracellular Na^+, the aim of the present study was to assess the contribution of Na^+ gradient to H^+-dependent HMTBA accumulation in chicken everted sacs.

MATERIALS AND METHODS

Materials

All reagents were purchased from Sigma (St. Louis, MO). Zoletil (tiletamine-zolazepam) was obtained from

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Table 1. DL-2-Hydroxy-(4-methylthio)butanoic acid accumulation in the intestinal wall of everted sacs (nmol/100 mg of tissue)[1]

	Presence of an H^+ gradient (n)		Absence of an H^+ gradient (n)	
Item	Presence of Na^+ (5 to 7)	Absence of Na^+ (3 to 4)	Presence of Na^+ (5 to 7)	Absence of Na^+ (3 to 4)
Duodenum	87.2 ± 7.5[a]*	65.4 ± 13.1[a,b]*	65.9 ± 6.1[a,b]*	62.0 ± 6.1[b]*
Jejunum	180.3 ± 5.8[c]	133.3 ± 10.8[d]	115.8 ± 12.7[d]	119.9 ± 13.5[d]
Ileum	192.2 ± 8.7[e]	144.9 ± 7.4[f]	115.5 ± 9.3[g]	99.5 ± 7.3[g]

[a–g]Mean values within the same row with different superscript letters are different ($P < 0.05$).

[1]The experiments were all conducted in parallel, although the data obtained in the presence of Na^+ were previously published in Martín-Venegas et al. (2006b). Results are expressed as mean ± SEM (numbers in brackets). Statistical comparisons were performed within a row (to compare within the different incubation conditions) or within a column (to compare within the different intestinal segments).

*$P < 0.05$.

Virbac (Carros, France). The HMTBA was supplied by Adisseo France S.A.S. (Antony, France) as Rhodimet AT88 (88% of active substance).

Birds and Diets

Male Ross 308 chickens (*Gallus gallus domesticus* L., Granja Crusvi, Montblanc, Catalonia, Spain) were raised at standardized temperature (26 to 28°C), humidity (40 to 60%), and light (16L:8D) at a density of about 1 chicken/500 cm^2. During the first week of life, the birds were maintained with an additional heat source. The birds were fed ad libitum from hatch to d 18 to 21 with balanced diets (IRTA-Mas Bové, Generalitat de Catalunya, Reus, Catalonia, Spain) supplemented with HMTBA as a source of Met, as previously described (Martín-Venegas et al., 2006a,b). The experimental protocol was approved by the Experimental Animal Ethical Research Committee of the Universitat de Barcelona, in accordance with the current regulations for the use and handling of experimental animals (Decret 214/97, Generalitat de Catalunya).

Transport Experiments

The chickens were anesthetized with 60 mg/kg of Zoletil and killed by decapitation without previous starvation. A portion of the duodenum (pancreatic loop), jejunum (6 cm proximal and distal to Meckel's diverticulum), and ileum (the region connected with mesentery to the caeca) was removed and immediately flushed with ice-cold saline solution (4°C). Everted sacs were prepared following Wilson and Wiseman (1954), as previously described (Martín-Venegas et al., 2006b). Each sac was filled with the serosal medium and incubated for 30 min at 37°C in 15 mL of the mucosal medium, which was continuously gassed with carbogen (95% O_2 and 5% CO_2). At the end of the incubation, the sacs were emptied, extracted overnight in 1 mL of HNO_3 0.1 Eq/L with continuous shaking, centrifuged (1,900 × *g* for 5 min), and stored at –80°C until quantification.

Sacs were incubated in the presence and absence of Na^+ (143 and 0 m*M*) in the mucosal compartment at a pH of 5.5 and 7.4. The mucosal medium was a modified Krebs-Henseleit bicarbonate buffer, which contained (in m*M*): 118 NaCl, 4.74 KCl, 1.18 $MgSO_4 \cdot 7H_2O$, 1.27 $CaCl_2$, 1.18 KH_2PO_4, 25 $NaHCO_3$, and 7 HMTBA, gassed with carbogen until pH 7.4. For the experiments performed at pH 5.5, $NaHCO_3$ was replaced by 2-(*N*-morpholino)ethanesulfonic acid, and pH was adjusted with Tris. The Na^+-free Krebs solution was prepared by replacing NaCl and $NaHCO_3$ with KCl and $KHCO_3$, respectively. In all the experiments, the serosal medium was the pH 7.4 buffer without substrate. Results were normalized to the weight of the empty sac after incubation and expressed as nanomoles/100 mg of tissue.

HPLC Analysis

The HMTBA monomer concentration was measured as previously described (Martín-Venegas et al., 2006b) in the Serveis Cientificotècnics of the Universitat de Barcelona, using a reversed-phase C_{18} HPLC analysis.

Statistical Analysis

The results are reported as means ± SEM. The data were compared by 2-sided Student's *t*-test using the SPSS statistical software package version 11.0 (SPSS Inc., Chicago, IL), and $P < 0.05$ was considered to denote significance.

RESULTS AND DISCUSSION

The Na^+ dependence of HMTBA transport was studied in everted sacs of the duodenum, jejunum, and ileum. This technique is a simple, reliable, and well-established in vitro model that allows the study of events at the apical membrane (Foulkes, 1996). The data of HMTBA accumulation in the intestinal wall obtained in the presence of an H^+ gradient show (Table 1) lower values in the absence of Na^+ along the chicken small intestine, although no significant differences were detected in the duodenum. In contrast, in the absence of an H^+ gradient, no significant differences were detected between the 2 Na^+ conditions. The regional profile observed in the absence of Na^+ is similar to that described in the presence

of this ion (Martín-Venegas et al., 2006b; i.e., the lowest values for the duodenum and no differences between the jejunum and ileum). The lack of Na^+ effect in the absence of an H^+ gradient confirms previous data of Brachet and Puigserver (1989), who reported that HMTBA transport was mediated by a Na^+-independent mechanism. However, the effect found in the presence of an imposed H^+ gradient indicates partial Na^+ dependence of HMTBA transport, mainly in the more distal regions, where the pH effect is stronger. This partial Na^+ dependence has also been described for other H^+-coupled transporters such as proton/amino acid transporter 1 and peptide transporter 1), and it has been attributed to the functional cooperation between these transport systems and NHE3 activity (Thwaites et al., 2002; Boll et al., 2004). This relationship has also been described for HMTBA transport in Caco-2 cells (Martín-Venegas et al., 2007). Therefore, we concluded that HMTBA uptake shows partial Na^+ dependence that reflects the participation of NHE3 in the chicken small intestine. Taking into account the importance of this analog in animal production, knowledge of the functional characteristics of the transport mechanism involved would allow studies concerning its regulation by dietary contents.

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